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Contributions to the Study of Subpopulations of Fishes

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EXPLANATORY NOTE

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The following collection of papers entitled "Contributions to the Study of Subpopulations of Fishes" is a written symposium by members and former members of the Branch of Fishery Biology. It is a substitute for a conference, thus lacking the discussion which is so often stimulating and which contributes to the development of a general consensus which did not exist among the group when the papers were written. The advantage of more leisurely preparation, of not having to meet a deadline, such as a meeting date, may outweigh the lack of debate, however, for although all of the men were engaged in active research, the subjects of some of the papers were collateral to the principal lines of work of some of them at the time of preparation. The papers were written voluntarily on invitation, rather than by assignment as an official function, and thus partake of the nature of contributions to some journal of marine fishery biology (now non-existent). If there is a spontaneous demand for a written symposium upon timely subjects in the future, the Special Scientific Report:--Fisheries, being of a less permanent or formal nature than the older printed publications of the Service, may offer its pages again under a rotating editorship for the symposium issues.

United States Department of the Interior, Fred A. Seaton, Secretary
Fish and Wildlife Service

CONTRIBUTIONS TO THE STUDY OF SUBPOPULATIONS OF FISHES

Coordinated by

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THE PROBLEM OF DEFINING AND RECOGNIZING SUBPOPULATIONS OF FISHES

By

John C. Marr[1/]

INTRODUCTION

In trying to identify and measure the causes of fluctuations in the abundance and distribution of a species of fish, it is essential that the number and identity of subpopulations, if any, within the species be established, since each subpopulation may have its own characteristic distribution, fecundity, natural mortality rate, growth rate, etc. This statement (or others like it) is practically axiomatic in the field of fishery biology; and yet, there is some misunderstanding arising in part from semantic difficulties and in part from the lack of agreed definitions of problems.

The semantic difficulties are those of the same term being used with different meanings by different workers, different terms being used with the same meaning, etc., often with no definitions given. The terms which I will use may be defined as follows:

Population: A population of fish includes all individuals of a given species when there are no subspecies or, if there are subspecies, when their distributions are not discrete. It includes only all individuals of a subspecies when the distributions of the subspecies are discrete. Obviously, there is gene flow, or opportunity for such, throughout a population.

Subpopulation: A subpopulation is a fraction of a population that is itself genetically self-sustaining. It is the smallest natural self-perpetuating unit and is synonymous with the term "deme" as used by systematists (Huxley, Evolution the modern synthesis, New York, 1942: 203). Although differences between subpopulations may be small, they are heritable.

Stock: A stock is a population or a portion of a population, all members of which are characterized by similarities which are not heritable, but are induced by the environment. A stock may or may not include members of several different subpopulations.

Group: A group is a fraction of a population with distinctive characteristics, the nature of which (phenotypic or genotypic) has not yet been determined.

Race: I prefer not to use this term, since in other fields (ornithology, for example) it is a synonym of subspecies. The categories involved in fishery problems are generally of lesser rank than a subspecies; if not, they should be recognized as such.

Although it might be desirable to standardize on this or some other set of terms, this is not essential so long as everyone defines whatever terms he does use.

The important distinction to keep in mind between subpopulation and stock is that members of a subpopulation segregate at spawning time, whereas members of a stock need not. It is also important to remember that members of a group actually are members of either a subpopulation or a stock, but cannot be assigned to one or the other category because of lack of information. The partial barriers to gene flow between subpopulations are of the same nature as those between subspecies and include isolation in space, isolation in time and ecological isolation.

The "subpopulation problem," then, involves first, the recognition and definition of each subpopulation and second, the planning of biological observations such that the data accrue discretely for each subpopulation rather than by groups of subpopulations. If observations are planned in such a manner that data accrue

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discretely for each stock, little if anything will be learned about subpopulations and, in fact, if the concepts of stock and subpopulation are confused the investigator may be very seriously misled. On the other hand, the characteristics of a stock may well serve as natural tags and be of great value in the study of availability phenomena. It is a fair generality to state that whereas most fishery biologists have been interested in defining subpopulations, they have, in fact, most frequently defined stocks or groups. This may be avoided only by using characteristics known to be genotypic or by using a more direct experimental method such as tagging.

METHODS

A number of diverse methods have been, or could be, used in subpopulation studies. Any classification or listing of the various methods must, perforce, be somewhat arbitrary rather than natural, since many of the categories overlap. The order in the following listing is suggested somewhat, but not entirely, by the historical order of their development.

I. Anatomical studies.

Various anatomical characteristics have frequently been used in an effort to distinguish subpopulations, much as they have been used to distinguish subspecies, species and higher categories. The types of characters used may be included in the following groups:

A. Morphometric: These include (1) external features such as the proportions of various body parts, (2) internal features such as the proportions of the various parts of the viscera and (3) cytological features such as the structure of various cells.

B. Meristic: These include (1) external features such as the numbers of fin rays and spines and (2) internal features such as the numbers of vertebrae and gill rakers.

C. Presence or absence of a morphological structure: The presence or absence of a particular structure such as teeth or pseudobranchiae may sometimes be diagnostic.

The use of anatomical characteristics in defining and describing subpopulations has at least two limitations. First, it is well known from both empirical and experimental evidence that body form, numbers of vertebrae, etc., are influenced by environmental variables (such as food and temperature, for example). Obviously, then, in using such characteristics the risk exists of studying the effects of environmental conditions rather than the effects of genetic isolation. Second, even if the characteristics used are genotypic, their frequency distributions generally overlap, often to a large degree. If significant differences are found between the samples, these have usually been interpreted as indicating that the two samples were drawn from two distinct subpopulations. Actually, this indicates only that they were not both drawn from the identical population; the possibility of considerable intermixing still exists.

II. Tagging or marking experiments.

Tagging or marking experiments have usually been carried out in order to learn about migrations or to make estimates of population size. They can also be used to learn something about subpopulations, provided that the tagging and recapturing are done on the spawning grounds.

III. Physiological characteristics.

Subpopulations may have distinctive physiological as well as anatomical characteristics. These might include, for example, inherent growth characteristics.

IV. Life history characteristics.

Differences in life histories may also be indicative of genetic differences between subpopulations. Such differences might include, for example, different spawning seasons or different temperature requirements at spawning.

V. Biochemical studies.

Recent advances in other fields are now being applied to fishery problems. They include:

A. Blood characteristics. The antigen-antibody reaction has been employed in studying differences (1) in blood sera and (2) in blood cells.

B. Muscle amino acids: Paper chromatography as a tool for identifying muscle amino acids has recently been used.

While these different techniques are rather exact and well known, they need extensive testing in order to determine whether or not differences observed through their use are a reflection of genetic differences.

VI. Genetic studies.

While difficult, it is conceivable that the direct approach of the geneticist in breeding experiments could be applied to marine fishery problems and, in the case of some fresh water species, it has, in fact, been so applied.

Some Recent Advances

The post-World War II intensification of interest in general fishery problems has, of necessity, included an intensification of interest in the subpopulation problem. As a consequence new techniques have been developed, techniques have been borrowed from other fields, previous studies have been critically reviewed and new studies have been carried out on fresh-water, anadromous and marine fishes. Examples of all these are included in the present collection and are briefly mentioned below.

Ordinarily comparisons of meristic or morphometric data have been made by tests of significance. These show, at some specified level of probability, whether or not the samples being examined could have been drawn from the same universe. Royce points out that the student of subpopulations (as opposed to, say, the student of species) is not so much interested in whether his samples were drawn from the same or different subpopulations, but rather in how much intermingling there is between the subpopulations represented by his samples. This is a very important distinction and one that has, perhaps, not previously been fully appreciated.

Because of the nature of their problems and data, fishery biologists have been reasonably quick to adopt statistical procedures as convenient tools for reducing data. Indeed, many statistical procedures have been modified or developed by fishery biologists seeking tools appropriate to unique needs. Thus, Royce has extended and modified previous methods so that it is now possible to simultaneously compare several characters and reach a maximum estimate of the amount of mixing that could occur between the populations sampled. It should be pointed out that this method indicates only the maximum mixing that could occur. It provides no information as to whether or not such mixing does in fact occur. Even so, this method will be of great aid to fishery biologists and will, I suspect, in some cases require extreme modification or even rejection of previously held hypotheses.

With a certain maximum amount of mixing possible, how can one determine how much mixing, if any, actually does obtain? As Royce points out, this information can be obtained from a tagging experiment. Under ideal conditions a tagging experiment could define the rate or percentage mixing. Unfortunately, these ideal conditions are seldom found and a tagging experiment may only show that some mixing does occur. Royce's method now enables us to set a maximum amount to the mixing that does occur.

A different and more restricted approach to the problem of detecting mixing has been taken by Widrig and Taft. They have considered the problem of determining the amount of mixing between two areas in some given time interval, say one year. The method they develop is such that if the mean value of some character is known for area A and area B at the beginning of the time interval and the mean value of the character is known for only area A at the end of the time interval, the percentage of fish in area A at the end of the time interval that came from area B can be determined.

As Widrig and Taft point out, there are a number of limitations to this method. Since the characters selected in practice may often be phenotypic, it will be necessary to consider only

a single year-class at a time. Furthermore, although it may be obvious that mixing is taking place, the number and location of the areas between which mixing is taking place will generally be much less obvious. In this connection, they point out that the power of this tool will be greatly increased by a concurrent tagging experiment, which would yield supplementary and complementary information.

In spite of these limitations, however, application of the method proposed by Widrig and Taft, should provide useful quantitative and qualitative information about availability changes in many fisheries.

Perhaps one of the most exciting potentialities in the study of subpopulations is the application of biochemical techniques borrowed from other fields. These methods are extremely promising because the characteristics which can be examined are either known to be genetically determined or can be examined experimentally to see if they are genetically fixed.

One of these techniques is the determination of tissue (muscle, for example) amino acids by the use of paper chromatography. Farris has briefly reviewed the method and some of the results that have been achieved to date. He also points out examples of non-genetic differences that have been observed under certain circumstances. Farris concludes that while systematic chromatography theoretically holds great promise, its general and specific applicability need to be critically evaluated. It might also be expected that the higher one goes along the chain of biochemical complexity, the more specific will the compounds become. For example, the occurrence of certain enzyme systems is more specific than is the occurrence of amino acids.

Another group of biochemical techniques, if they may be so termed, are those borrowed from the field of immunology. Ridgway has reviewed blood characteristics which may be useful in the identification of subpopulations. These include (1) differences in red cell antigens, (2) naturally occurring hemaglutinins and (3) the antigenic properties of serum proteins.

Of these three possibilities, the hemaglutinins hold the least promise, since they are of irregular occurrence and doubt has arisen over their genetic determination. The antigenic properties of blood serum are somewhat more promising, although intraspecific differences have only rarely been demonstrated. Ridgway suggests that the application of the agar diffusion technique may be useful here. The use of serum has advantages over the use of blood cells in that serum can be easily frozen and held for long periods. On the other hand, the technological problems associated with preserving red cells have not yet been satisfactorily resolved. Nevertheless, the use of red cell characteristics holds the most promise among the immunological possibilities of attacking the subpopulation problem. Differences in the antigenic composition of red cells have been shown to be genetically controlled wherever they have been examined by the appropriate methods. Furthermore, individual differences have been found among diverse groups of animals.

Thus, the requisites of a useful tool for the study of subpopulations seem to be found in the red cell characteristics. The main problems in this regard are those involved in the application of the technique to specific situations.

After briefly considering new techniques and techniques borrowed from other fields and before reviewing recent studies that have been made of particular species, it is instructive to examine in retrospect some previous studies of the subpopulations of several species of fishes. Ahlstrom has done this for eight Pacific species, including one clupeid, four engraulids and three scombroids.

For all of these species the meristic and/or morphometric approach has been used. Interestingly, meristic studies have been largely confined to the clupeid and engraulids, while morphometric studies have been made of the scombroids. None of these studies have been definitive, owing to the inability of the investigators to distinguish between phenotypic and genotypic characters. In two studies, those of the Pacific herring and Pacific mackerel,

data from tagging experiments were also available. The tagging data require considerable modification of conclusions based on meristic or morphometric data alone (as Royce has discussed in some detail).

Ahlstrom concludes from his review that, while the indirect meristic and morphometric studies generally show heterogeneity within a population, only direct methods will demonstrate the actual amount of intermingling. Carefully designed tagging experiments are indicated as the solution to this dilemma.

Turning now to some recent studies, Cope has had the opportunity of studying cutthroat trout in Yellowstone Lake. This subspecies has long been naturally isolated (and no introductions have been made) in a situation offering a diversity of ecological habitats and, presumably, a like amount of opportunity for micro-evolution.

This trout ordinarily inhabits the lake and only ascends tributary streams to spawn. The trout spawning in five of these tributaries were examined. There was a marked tendency for adult fish to return to the same stream in which they had previously spawned. (Young downstream migrants were not marked for homing studies). In addition, between-stream differences were observed in the time of the spawning migration, the size and age composition, and the size and number of eggs produced.

Cope has accepted these differences as evidences of the existence of at least five distinct subpopulations of cutthroat trout in Yellowstone Lake. Since there is no experimental evidence, it might equally well be argued that the only characteristic which is heritable is that of homing to the natal stream (whichever it may be) and that all the other differences are phenotypic differences imposed by the natal stream Even if this perhaps extreme view should prove to be true, nevertheless as long as the fish do show persistent homing there is an effective method of isolation operating. If the fish of different streams have not yet genetically diverged, it may be expected that they will eventually do so under the selection of the differing environments inhabited during at least part of their lives. The nature of this problem and of the natural habitat of the fish suggests interesting experiments that could be done in situ.

Raney has continued his studies of the striped bass, considering here the possible existence of subpopulations in Chesapeake Bay and its tributaries. He concludes that there are three subpopulations, one in the James River, one in the York and Rappahannock Rivers and one in the bay and tributaries north of the Rappahannock. He also believes the James subpopulation to be differentiated at a higher level than the others.

His study is based on meristic characters, including dorsal spines and dorsal and anal rays. The recognition of subpopulations on this kind of evidence requires that assumptions be made about the genetic nature of the observed differences. Raney has made these assumptions, yet he clearly recognizes that they are assumptions and has gone to some length to examine their validity, or at least to remove some of the extrinsic variables. To this end he has examined only fish of the year which were collected at or near their place of origin, he has considered characters by year-classes and has followed them through several year-classes, he has considered characters over wide geographic areas and he has drawn on supplementary sources of information such as the results of tagging experiments. Definitive and final answers to this problem must, as Raney suggests, await experimental evidence.

In reviewing the subpopulation problem in the Pacific sardine, I have attempted to gather all available evidence in an effort to clearly define the problem and to suggest methods of attacking it. The single most important body of information in this respect is the data on the space-time distribution of spawning and on the absolute magnitude of each space-time group (of which there are at least four).

The crucial problem is, I believe, to determine the amount of interchange between these space-time groups. This obviously imposes the requirement of sampling on the spawning grounds. The more direct the attack on this problem the more definitive will be the results. A tagging

experiment is the most direct method, but is costly. On economic grounds the possibilities of other methods, such as certain immunological techniques, should be examined first.

I have also included a tagging experiment design which will be of use in sardine studies and, perhaps, be of broader application.

Snieszko has taken a somewhat novel approach to the problem of defining subpopulations and has done so on the basis of differences in disease resistance and susceptibility. He considered experimentally the effects, in terms of mortalities, of furunculosis and/or ulcer disease on brook trout from different localities. Some of the infections occurred "spontaneously" and some were induced. Trout from two localities showed very high, although not identical, mortalities, while trout from a third locality showed very low mortalities.

The implications of these studies extended to the field are obvious and a broader application of this experimental approach would be of great interest. The explanation for some anomalies in fish distribution may, in fact, be found in an earlier work of Snieszko's in which he showed that disease resistance in fish (antibody formation) varied with temperature.

An interesting and important problem, but one perhaps not strictly pertinent here, is an evaluation of the importance of pathogens as a source of fish mortality in nature; especially of marine fishes.

Finally, in this collection, Eschmeyer has called attention to a problem that has received little attention; namely, the possible existence of subpopulations of lake trout in the Great Lakes. The evidence which suggests that there are subpopulations of lake trout includes differences in spawning time, spawning substrate, spawning depth, size at maturity and fat content. Also, tagging evidence indicates the existence of a homing tendency. Study of this problem may be particularly urgent in view of predation upon lake trout by the lamprey.

CONCLUSIONS

The preparation of this collection, insofar as I have been responsible for it, has been an extremely instructive process. To the extent that the papers in this collection are representative of the studies now being made of subpopulations, some generalizations about the present status of such studies may be made.

First, there appears to be a reasonably widespread realization that the classical meristic-morphometric approach alone will not lead to a satisfactory and conclusive solution of this problem.

Second, and perhaps as a corollary of the first, the genetic implications of this problem are coming to be more generally realized and accepted.

Third, there seems to be more effort devoted to developing new and more satisfactory tools and to borrowing and adapting pertinent tools from other fields.

Fourth, the desirability of a direct attack (tagging) on this problem is being recognized by more and more individuals.

Finally, and perhaps to a lesser extent, the applicability of the experimental method to problems in the field is being appreciated.

If these generalizations are valid, we may look forward to an increased rate of progress and understanding in this field.

STATISTICAL COMPARISON OF MORPHOLOGICAL DATA

By

William F. Royce[1/]

Taxonomists concerned with the smaller taxonomic units in all fields have used a great variety of quantitative methods together with various kinds of qualitative evidence to arrive at decisions on the relationships of species and subspecies. Generally, the quantitative methods have been sparingly used to substantiate a conclusion already reached from the qualitative evidence. We may infer that the qualitative evidence has usually been considered adequate and that quantitative methods are not always necessary. With small forms which may be arranged in a laboratory and directly compared it is easy to find the best characters which will distinguish groups one from the other, and if clear differences are found, then no statistical methods are needed nor desirable.

If, on the other hand, it is not possible to see and compare many specimens at one time, it is necessary to quantify the characters, and the search for the best characters become much more difficult. The often subtle evidence of allometric or isometric growth must be sought before considering a character. The multiple sensory impressions that are so useful when things are compared side by side become uncertain and unreliable. Such a situation has confronted biologists who have been concerned with the races of tunas a group in which it is not possible to compare directly most of the specimens. It has been necessary to guess at the distinguishing characters, to measure them in the field, and to compare them statistically in the laboratory.

Then when the data are at hand the usual statistical methods in current use by fish taxonomists are not adequate It is not possible to compare directly frequency distributions because the characters used in this group have been mostly the size of body parts, which are of course related to the size of the fish. Such measurements usually have been compared as ratios, but ratios too are related to the size of the fish because of the prevalence of allometric growth. Even the regression analyses which have been used especially for tunas have not fully allowed for the complex allometric growth. Then there has been a tendency to make comparisons by means of tests of significance, a method which does not make full use of the data. Neither has there been a satisfactory method of comparing two groups while using more than one character at a time.

Tuna biologists also have an important objective in addition to the usual taxonomic ones. They want to know the amount of intermingling of the stocks of tuna, not just whether they are distinct enough to deserve different specific or subspecific names. Studies based on taxonomic principles provide answers to these problems that supplement direct evidence of intermingling.

I have prepared this paper with the special objective of developing methods of analysis suitable for racial studies of tunas. It will also have obvious applications to intraspecific studies of many groups, especially those in which measurement data are used. In it I will briefly review the quantitative methods in current use. These include averages, ratios, and the more precise regression analysis. I will (1) consider the relative merits of the test of significance and the measurement of overlap, (2) discuss how to determine overlap of counted characters and show how to find the overlap of measured characters through regression analysis, and (3) show the relation between overlap and a concept of the distance between the means and then apply a generalized distance function to measure simultaneously the overlapping of several characters.

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TAXONOMIC CHARACTERS

Any morphological character may be expressed either as a count or as a measurement. Counts are made of the number of body parts: fin rays, vertebrae, scales, etc. The presence or absence of a part, e.g., a barbel, fin, or scales on a certain part, may be considered as a special kind of count which is either 1 or 0. All other things may be measured: distance, area, volume, weight, angle, etc. We shall not be concerned with non-morphological characters, but such things as color, physiology, and fertility may usually be measured too.

In any search for distinctive characters, we seek those which reflect inheritable genetic differences regardless of sex, size, nutrition and environment. If a character is a function of any of the latter factors, then we may either compare samples which are identical with regard to these factors (e.g., all the same sex) or introduce a mathematical adjustment that removes the non-inheritable difference. In this paper we should be concerned with characters that are related to size, but it should be noted that the other factors may be considered in similar ways.

Any morphological character may be a function of total size, but usually counts are not and they are generally used as though independent. On the other hand, measurements of fish almost always are related to body size. This makes it obvious that the effect of body size must be eliminated, and fish taxonomists usually have attempted this by using ratios. However, ratios are difficult to use (Marr 1955) because parts are seldom related to body size in a simple constant ratio. This has led Ginsburg (1939) to doubt the value of measured characters (distances) and Parr (1949) to propose a relatively complicated formula for dealing with them.

Another approach was made in the first studies of the morphometric characteristics and relative growth of yellowfin tuna by Godsil (1948) and by Schaefer (1948), who used regression analysis to describe the size of body parts. Both of these authors found that body proportions or ratios of the size of a part to total length were unsatisfactory except in the rare instance where the ratio had a constant relation to the total length or, in other words, where the growth of the part was exactly proportional to the growth of the fish. When the data are subjected to regression analysis this is the unique case in which the regression line is straight and passes through the origin. Godsil found that not only did the regression lines rarely pass through the origin but that in most cases when a large amount of data was available a slight curvilinearity was obvious, and he fitted his regression lines with the formula

$$\hat{Y} = a + bX + \frac{c}{X} \qquad (1)$$

in which a, b, and c are constants, X is the fork length of the fish, and $\hat{Y}$ is the estimated length of the character measured. Schaefer, on the other hand, found a satisfactory fit using a straight-line regression method for most characters

$$\hat{Y} = a + bX \qquad (2)$$

but with the length of the pectoral, second dorsal, and anal fins he found it necessary to transform either the fin length or fork length or both to logarithms in order to obtain a sufficiently straight regression line. Marr (1955) points out that transformations are usually satisfactory and are much easier to analyse than curvilinear regressions.

TESTS OF SIGNIFICANCE

After determining the regression line which gives a satisfactory fit, most authors have used a test of significance to decide whether two or more samples could have been drawn from the same population. Godsil (1948) compared Central American, Japanese, Hawaiian, and Peruvian yellowfin and found that with one or more characters there were highly significant statistical differences between areas. Schaefer and Walford (1950) found similar differences between yellowfin of Central America and Angola, Africa. These methods have been followed in subsequent papers, all of which indicate highly significant statistical differences between areas. Such statistical differences have been found so consistently that Royce (1953) concluded that, even with samples from closely related stocks, highly significant statistical differences could always be found by increasing the size of the sample, by considering enough characters, or

by applying the more critical discriminant function proposed by Fisher (1936b). A similar conclusion has been reached by taxonomists in other groups, for according to Mayr, Linsley, and Usinger (1953:151) "the mere fact of a (statistically provable) difference between several populations of a species is therefore of no special interest to the taxonomist; he takes it for granted. Even the lowest recognizable taxonomic category (the subspecies) is normally composed of numerous populations that differ 'significantly' in gene frequencies and in the means of certain variates."

Thus the conclusion that a statistically significant difference exists between samples becomes a trivial one. It is a necessary preliminary in racial studies, but once it is found that significant differences can be expected from the samples that are most closely related in time and space it is no longer useful. As Fisher (1936a) has pointed out, a test of significance is merely a means of making a decision, and once the predetermined level of significance is reached, larger samples and further sampling merely reiterate the conclusion. A test of significance[1/] decides that there is a difference, and after it is found we become interested in the quantity and direction of the difference.

A step toward determining the quantity of the difference has been employed by Godsil (1948) and by Schaefer (1955), both of whom have determined that differences between samples from widely separated areas are greater (less likely to occur due to chance) than the difference between samples taken close together. Godsil, using a modified analysis of covariance, showed a much greater difference in the mean square when comparing distant samples than when comparing "local" samples. Schaefer (1955), using conventional analysis of covariance techniques, came to a similar conclusion with regard to Central American and southeast Polynesian yellowfin. Such analyses have shown merely that the differences are much greater but not how much in units that can be readily compared.

1/ Further discussion of a test of significance will be undertaken in the sections on sampling problems and comparison of tagging and morphometric data.

Another method which gives a direct comparison has been used by Royce (1953), who computed the size of various body parts for yellowfin of a given total length. He showed there was an average difference of 1.6 cm. in the head length of a yellowfin tuna 100 cm. long as between the western Caroline Islands and Costa Rica and that the samples of tuna from the intervening areas along the Pacific Equator had head lengths of intermediate sizes suggestive of a cline from east to west A reverse cline was apparent in the height of the anal fin, with differences of up to 7.2 cm. between Costa Rica and the Caroline Islands. This method provides a ready means of comparison, but it still does not consider the amount of intergradation or overlap.

CONCEPT OF OVERLAP

Taxonomists in many fields have used the degree of overlap in studies of inter- and intra-specific variations. Among fish taxonomists, Ginsburg (1938) postulated that the best means of comparison was the extent of intergradation or the amount of overlapping of principal characters. He gives many examples of meristic or countable characters, and he compares samples by the actual overlap of the percentage distributions. This he computed as a percent (p*) obtained from the sum of the smaller percents (%) of the frequency classes in the two samples in the area of overlap.

$$p^* = \frac{\sum (\%1 < \%2) + \sum (\%2 < \%1)}{2} \qquad (3)$$

Other taxonomists have computed the mean and standard deviation of such frequency distributions and from them determined a single figure for the distance between the populations which is directly indicative of the amount of non-overlapping. This figure is simply the absolute value of the difference between the means ($\bar{x}_1$ and $\bar{x}_2$) divided by the summed standard deviations of the two populations (σ_1 and σ_2)

$$C.D. = \frac{|\bar{x}_1 - \bar{x}_2|}{\sigma_1 + \sigma_2} \qquad (4)$$

It is called a coefficient of difference (C.D.) by Mayr et al (1953:146), who give it in a slightly different notation.

The fundamental difference between these two methods of computation is one of comparing samples or comparing estimates of population parameters. In Ginsburg's method a simple comparison of samples is made and no precise mathematical inferences about the populations are possible. In the other method it is assumed that the samples have been obtained from normal distributions and precise mathematical inferences about the populations may be made. In addition it becomes possible to use the immense background of mathematical and statistical experience which has been concerned with normal populations.

We prefer to change slightly formula (4) to a form which will be a starting point for the generalization which follows. It becomes

$$D = \left| \frac{\bar{x}_1 - \bar{x}_2}{\bar{\sigma}} \right| \qquad (5)$$

in which $\bar{\sigma}$ is the pooled within-sample standard deviation computed from the pooled variance of the two samples and D is the distance between the means in the standard measure of statistics, i.e., in units of the standard deviation. It will be obvious that

$$C.D. \sim \frac{D}{2}. \qquad (6)$$

The difference arises because in (5) the average standard deviation is computed from the pooled variance, which is the usual statistical way of estimating the standard deviation in the population.

A graphic presentation of the normal frequency distribution will illustrate this concept. If we simplify the illustration by assuming large sample size and equal variance[2/] with D = 2.5, then the plotted normal frequency distributions are as in figure 1. The area of overlap is shaded. It may be seen that an individual from one of the two samples with a character of size $X = \frac{\bar{x}_1 + \bar{x}_2}{2}$ will have equal probability of being correctly classified on the basis of the character. As X becomes greater the probability increases that the individual "belongs" to population 2 and as X becomes smaller the probability increases that the individual "belongs" to group 1. Since, in any normal distribution, X may be infinitely large or small the probability of correct classification never reaches 1 but soon approaches close enough for practical purposes.

Instead of considering an individual having a particular character X we may consider all individuals in which $X > \frac{\bar{x}_1 + \bar{x}_2}{2}$ or those in areas 2A and 1B[3/] in figure 1. It will be obvious that the area of 1B is equal to 2B with our simplifying assumptions, and hence we may use the relative areas 2A and 2B to determine a probability. The area corresponding to 2A may be obtained from a table of the normal probability integral, such as Pearson's (1948, table 2), if the samples are large, i.e., $n_1 + n_2 > 30$. In this table is given the area of half of the normal curve plus the space from the mean to the argument, $x = \frac{D}{2}$. We shall call this relative area the probability 1 - p.

If the samples are small and greater accuracy is desired, it is necessary to use a table of t, such as table 3 in Fisher and Yates (1948). When this table is entered with the arguments $t = \frac{D}{2}$ and $n_1 + n_2 = n$, a probability P_t is found, which refers to both tails of the distribution. Our p defined above may be found by

$$p = \frac{P_t}{2}. \qquad (7)$$

Despite its increasing use, considerable confusion exists regarding the name of the phenomenon and the exact meaning of the probability figure. Mayr et al. (1953:146) call 1 - p

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2/ It should be noted that it is not necessary to have equal sample size for the method to be valid, because the means and variances are practically independent of sample size. It will be shown later that moderate departures from equal variance are also permissible.

3/ Area 2A includes all of the area of distribution 2 greater than $\frac{\bar{x}_1 + \bar{x}_2}{2}$, area 1B all of the area of distribution 1 greater than $\frac{\bar{x}_1 + \bar{x}_2}{2}$. Hence, 2A and 1B are overlapping.

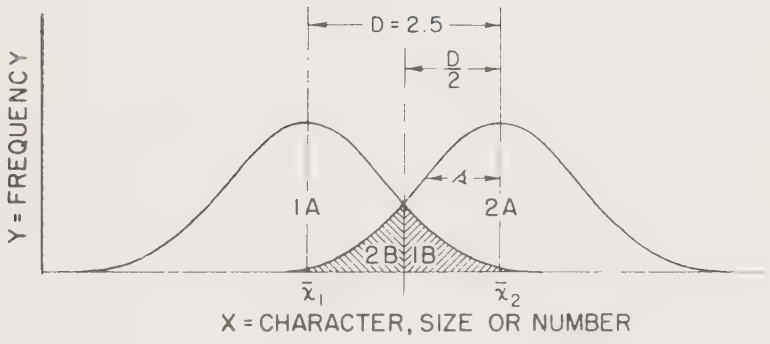

Figure 1.--Overlap of normal distributions.

(for example area 2A in fig. 1) the joint non-overlapping; Ginsburg (1938:255) calls it a measure of divergence; Mather and Dobzhansky (1939:15) use p and consider it the frequency of misclassification. To call it the amount of overlap is troublesome because as

$$D \longrightarrow O$$
$$P \longrightarrow 0.5$$
and
$$1 - p \longrightarrow 0.5.$$

A condition of complete overlap is indicated by

$$p = 0.5.$$

It is probably simplest to consider p as the probability of misclassifying an individual from one of the two samples by use of the character in question. The value of 1 - p is the probability of correct classification and varies from .5 or 50 percent with complete overlap to nearly 1 or 100 percent with no overlap.

There is, however, a need for another concept of overlap. This we define as the proportion of one sample which might "belong" to another specified sample. We consider it to be the entire area under one curve which is also included in the other. We assign to this concept the Greek letter omega Ω and express it as a percentage. It will be obvious that under the restrictions of equal sample size and equal variance (fig. 2) $\Omega = 200p$

This concept has an obvious advantage over p in some applications because a condition of complete overlap is indicated by 100 percent; in terms of our definition all of one sample might belong to the other. It also is a complete departure from the concept of the probability of misclassifying an individual (which with no information at all would be misclassified only half of the time) to the concept of the proportion of a sample which might belong to another group. From the samples, if they are representative, we may then make inferences about the populations.

USE OF OVERLAP

A concept of overlap has been used along with other data on geography, ecology, or qualitative characters in the definition of species and sub-species, but there is no general agreement on the meaning of different amounts of overlap (Edwards 1954). Ginsburg (1938), whose concept of overlap p* was similar to our p, used the overlap of frequency distributions to show a continuous gradation from p = .5 in two samples of the same species to no overlap in two separate but closely related species of the same genus. He suggested that a p = .1 would commonly be found between species, .3 between sub-species, .4 between races, and up to .5 between varieties. Mayr et al. (1953) suggest that the conventional level of sub-specific difference is p = .1, Ω = 20 percent, that is, a difference between the means of 2.56 times the average standard deviation (D = 2.56). Hubbs and Hubbs (1953:56) state that the more usually accepted sub-specific difference amongst fishes is equivalent to p = .25, Ω = 50 percent. It is evident that the amount of overlap is a continuous positive distribution and the level used for a decision must be chosen arbitrarily or in association with other factors.

Comparisons of overlap computed from the estimates of the population parameters (formula 5) with overlap computed directly from the samples (formula 3) indicate that when large normally distributed samples are considered the two methods yield nearly identical results. To show this we shall compare p* and p using data selected from published material to include a range of values of D and to include some material which we shall refer to later in this paper.

An example of overlap near the borderline of subspecific differentiations is given by Ginsburg (1938:269) as the frequency distribution (table 1) of the articulate dorsal rays in the weakfish Cynoscion regalis regalis from the Atlantic coast of the U. S. and Cynoscion regalis arenarius from the Gulf of Mexico. A summation of the smaller percentages (formula 3) provides an estimate of overlapping of

$$p^* = \frac{2.63 + 13.68 + 18.49 + .84}{2} = 17.8\%.$$

Substituting the values of the means and pooled standard deviation in formula 5 we have

$$D = \frac{27.258 - 25.874}{.8221} = 1.683$$

$$\frac{D}{2} = .841$$

$$p = .20 .$$

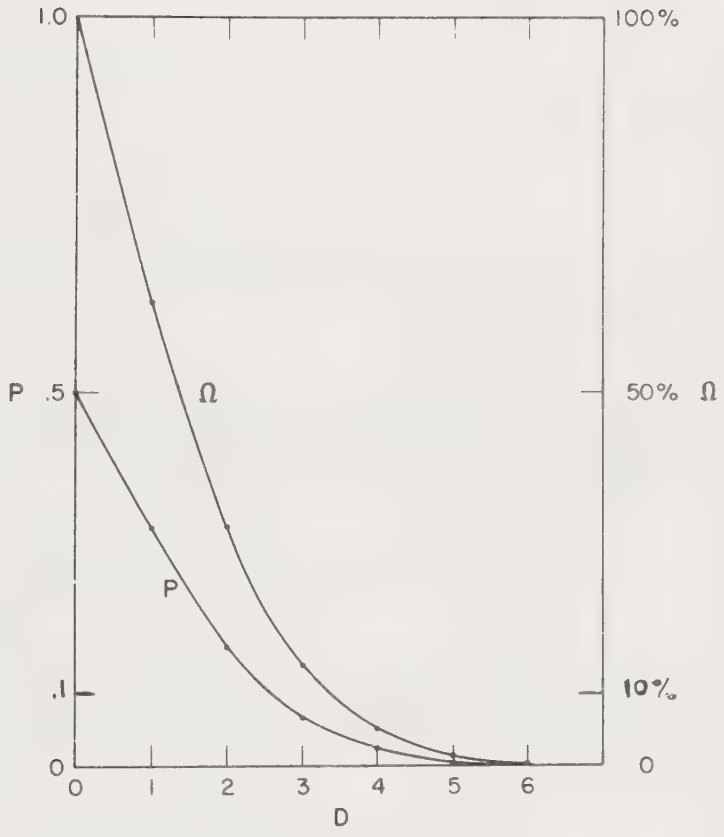

Figure 2.--Relation of the distance between means D to the probability of misclassification p and overlap Ω.

Table 1.--Frequency and percent distribution of articulate dorsal rays of weakfish Cynoscion regalis, from Ginsburg (1938:269)

Area	Number of dorsal rays						Total
	24	25	26	27	28	29	
Atlantic coast							
Number	-	5	26	85	63	11	190
Percent		2.63	13.68	44.75	33.16	5.79	100.01
Gulf coast							
Number	2	35	59	22	1	-	119
Percent	1.68	29.41	49.58	18.49	.84	-	100.00

A condition of nearly complete overlap was found in vertebral counts from samples of herring off Vancouver Island by Tester (1949). He compared five areas using large samples and found highly significant statistical differences among them. The greatest difference, that between the northernmost and southernmost areas, showed overlap by summation of the smaller percentages of

$$p^* = \frac{.57+21.22+60.35+11.43+24+.03}{2} = 46.9\%.$$

A very similar result comes from the difference between the means divided by the pooled standard deviation,

$$D = \frac{51.943 - 51.830}{.639} = .177$$

$$\frac{D}{2} = .088$$

$$p = .465$$

An excellent method of graphical presentation of morphological relationships has been proposed by Hubbs and Hubbs (1953). This is followed in figure 3 for the two sets of data discussed above. The bars show all of the pertinent statistics from the two samples, the mean ($\bar{x}$), twice the standard error of the mean ($2s_{\bar{x}}$), the standard deviation (s), and the range. These values provide a ready comparison, for when the hollow bars just meet ($D \approx 2$), a .16 level of overlap ($p(\Omega$ equals 32 percent) is indicated, and when the solid bars just meet, the means are just about significantly different. The solid bar also indicates approximately the 95-percent fiducial limits of the mean. Thus, as the authors point out, the measures of reliability and the measures of dispersion are both indicated.

Since both the standard deviation (s) and the standard error ($s_{\bar{x}}$) are based on the normal probability function, it is necessary to assume that the data are normally distributed, and if precise comparisons are needed the sample variances of the two samples to be compared must not differ from each other more than would be expected by chance. In other words, if the null hypothesis is used it is the hypothesis that the two samples are randomly drawn from the same normal population.

Such an assumption, even though not proved true, will not invalidate our use of the method. This matter of non-normality is one which has bothered all statisticians; Cochran (1953:22) gives a good discussion and in general says that no completely safe rules have been found but that the distribution of the means tends toward normality as the sample size increases in many highly non-normal distributions. In small samples from moderately skewed distributions empirical studies have shown that the statistics depart only a negligible amount from normality. However, this is a problem which each taxonomist will want to explore with regard to the data with which he is working.

Another problem, more frequently encountered, is the one of different variance. Here again, if only approximate results are needed it may be ignored; if precise results are necessary then tests and corrective formulas may be found in many statistical texts. However, as will be discussed later, if heterogeneous variance is present it may be evidence that population differences exist or that sampling methods have been inadequate or faulty.

OVERLAP OF MEASURED CHARACTERS

Only a simple extension of the method of determining overlap of counted characters by the difference between the means is required to determine the overlap of measured characters if regression analysis has been used. Instead of a mean we use a mean value for the character estimated from the regression line at a given body length at or near the grand mean length. Instead of the pooled standard deviation we may use the pooled standard deviation from the regression[4/] lines. Formula (5) becomes

$$D = \frac{\hat{Y}_1 - \hat{Y}_2}{\sigma_{y.x}}, \qquad (8)$$

4/ The standard deviation from regression is also frequently called the standard error of estimate. However, we refer here to the distribution of individuals around a line, and standard error in another usage refers to a distribution of means around a grand mean. Hence we avoid the latter term.

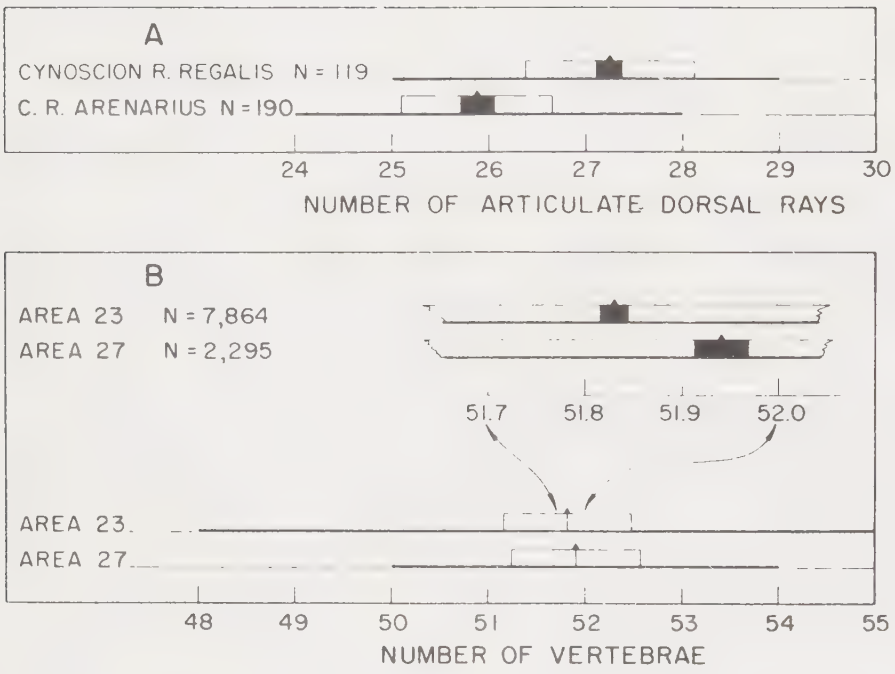

Figure 3.--Examples of overlap. A from Ginsburg (1938) showing the approximate level of subspecific differentiation in Cynoscion. B from Tester (1949) showing very closely related samples of herring from Vancouver Island which had statistically significant differences in mean numbers of vertebrae. The base line indicates the range, the small triangle the mean $\bar{x}$, the solid bar two standard errors $2s_{\bar{x}}$ on either side of the mean, and the hollow bar one standard deviation s on either side of the mean.

in which $\hat{Y}_1$ and $\hat{Y}_2$ have been computed from their respective regression formulae for the same value of X.

The overlap might be computed from ratios, but as Schaefer (1948 et seq.) and Marr (1955) have pointed out, the prevalence of heterogenic growth in the body parts of fishes makes the use of ratios inefficient and has frequently even led to erroneous conclusions. If heterogen ic growth is present, the ratio changes with the size of the fish, sometimes in a more complicated mathematical relationship than the direct relation of size of body part to size of body. For simplicity and for precision it is desirable to use the regression of the measured part on length of fish or of some other part.

A more precise answer may be obtained, if desired, by using a refined estimate of the standard deviation from regression. Such a refinement is necessary when the value of X is not the mean value, and hence the estimate of Y is subject to the variance of the slope of regression as well as the variance around the mean The appropriate adjustment (Snedecor 1946:137) is

$$s_{y.x}\sqrt{1 + \frac{1}{n} + \frac{(X - \bar{x})^2}{\sum (X - \bar{x})^2}} \qquad (9)$$

in which $(X - \bar{x})$ is the difference between the mean and the assumed X and $\sum (X - \bar{x})^2$ is the summation of all deviations squared from the mean $\bar{x}$. Our use of the unadjusted $s_{y.x}$ is thus justifiable only when our assumed X s near the mean when $\frac{(X \quad \bar{x})^2}{\sum (X - x)^2} \longrightarrow 0$ and in large samples when $\frac{1}{n} \longrightarrow 0$. The cor responding formula (Snedecor 1946.137) for the standard deviation of the estimated mean is

$$s_{\hat{y}.x} - s_{y.x}\sqrt{\frac{1}{n} + \frac{(X - \bar{x})^2}{\sum (X \quad \bar{x})^2}} \qquad (10)$$

An example of overlap of a measured character may be computed from the data on Pacific mackerel given by Roedel (1952). He compared the head lengths in samples from off California and Baja California nd found highly significant differences between the regions hich he called California, Soledad, Viscaino, Cape, and Gulf. The mackerel in some of these regions had been tagged several years earlier, and since we want to compare morphometric studies and tagging results later in this paper, we shall select the regions from which tagging studies are available.

The first comparison will be between California and Soledad. Using data from Roedel's table 5, the estimated head lengths for a total length of 250 mm. (near the grand mean) are 67.47 and 67.76 mm. The pooled standard deviation from regression is 1.234 mm. Substituting in formula (8) we have

$$D = \frac{67.76 - 67.47}{1.234} = .235$$

$$\frac{D}{2} = .118$$

$$p = .453$$

$$\Omega = 90\%$$

which is a condition of nearly complete overlap.

We will also need a comparison of California and Viscaino. The estimated head length for the latter at 250 mm , fork length is 68.68, and the pooled intragroup standard deviation from regression is 1.532. Using similar computations, we find that $p = .347, \Omega - 69\%$, which indicates considerably less overlap.

In order to show how this method of estimating overlap compares with Ginsburg's method for counted characters, we have modified Ginsburg's method to compute the overlap between California and Soledad. Since Roedel (1952) found a significant difference in the regression coefficients from these two regions, a comparison at 250 mm. will not be valid at all sizes. Therefore, we have selected fish from 200 to 299 mm. fork length and determined the difference from the joint regression line for the head length of each individual from each region (table 2). The measure of overlap is

$$p^* - \frac{86 + 10\ 34 + 21.55 + 31\ 71 + 12\ 20 + 9\ 76}{2} - .432,$$

a val e which is in very good agreement with $p - .453$ computed by the other method. W consider, therefore, that with large samples which are nearly normally distributed both methods

Table 2.--Data for computation of Ginsburg's measure of overlap in the head length of Pacific mackerel from Roedel (1952)

Region	Deviations from joint regression in mm.							Total
	-3	-2	-1	0	+1	+2	+3	
California								
Number	1	12	25	49	23	6	-	116
Percent	.86	10.34	21.55	42.24	19.83	5.17	-	99.99
Soledad								
Number	3	6	10	13	5	4	-	41
Percent	7.32	14.63	24.39	31.71	12.20	9.76	-	100.01

give similar results, but Ginsburg's method requires more computation for measurement data.

It is entirely practical to modify the method of Hubbs and Hubbs (1953) in order to illustrate the overlap of measured characters when regression has been used. The Pacific mackerel data are good material, and we may include the two southern regions not discussed above. By plotting the estimated mean head length for a fork length of 250 mm. we have a value corresponding to the mean of a counted character. Then the standard deviation from regression $s_{y.x}$[5/] may be plotted around the mean as a hollow bar corresponding to the standard deviation (fig. 4). Twice the standard error of the estimated mean may be shown as the solid bar on either side of the mean in order to show the reliability of the mean. The solid base line indicating the range (maximum deviation above and below the regression line) could be included too, but it is laborious to compute. The two bars that show the reliability and the overlap are the most useful statistics and together with the estimated mean length will provide the desired comparisons.

The accuracy of the overlap computed from the difference between means along regression lines depends on certain assumptions, specifically: that the regression equations are the best fitting ones; that the distributions about the lines are normal, homogeneous among regions, and not related to the size of X. Furthermore, if the regression coefficients are different, the lines will cross and the overlapping obviously will depend on the distance from the crossover.

Some of these requirements are not met. The plotted data in Roedel's (1952) figure 4 give no reason to suspect curvilinearity or non-normal distribution, but the distributions clearly spread out as fish become larger (the standard deviation is related to the size of X). Neither are the variances homogeneous among regions; the standard deviation from regression varies from .92 for Soledad to 1.79 for the Gulf. The author also shows that the regression coefficients differ significantly.

A spreading out of the distributions as the fish become larger is expected in measurement data of this kind. Consequently $s_{y.x}$ is an average which should be a good estimate of the dispersion of points near the mean. Hence it is another reason to compute the overlap for values of $\hat{Y}$ near the mean.

Some relation of length to $s_{y.x}$ is evident in the mean values of $s_{y.x}$ plotted in figure 5, but it is not a close relation. The three lower values are almost exactly proportional but the other two are higher than would be expected on the basis of the change in mean length. The greatest discrepancy is $s_{y.x} = 1.68$ for the Viscaino fish, which average 278 mm. in length, whereas the expected $s_{y.x} = 1.28$, if we assume that the $s_{y.x}$ is in the same proportion to mean length as in the samples with the three lower values. Squaring these and using the simple F test for the homogeneity of variance described by Snedecor (1946:248), we find

$$F = \frac{2.822}{1.632} = 1.72$$

when

$$F_{.05} = 1.47$$

This is the extreme example, but the probability against so large a difference occurring by chance is large enough to indicate some heterogeneity not associated with differences in mean length.

All of these problems, the association of $s_{y.x}$ and mean length, the greater heterogeneity in some samples, and the difference in slope of regression lines, will interfere with either tests of significance or estimates of overlap. We can minimize the effect of the first by making estimates of overlap for values near the mean. The effect of the second will be minimal if the intragroup $s_{y.x}$ is computed separately for each comparison. As for the last, a small difference in slope may be ignored without losing information. If it is large, however, it may be desirable to seek the point of minimum overlap in order to

5/ The refined formula (9) causes a 5-percent increase in the standard deviation from regression for the Gulf sample but no change through the second decimal place in the other samples. It is not used in figure 4.

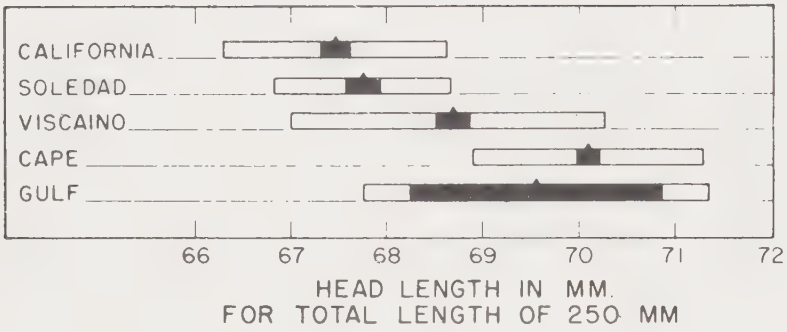

Figure 4.--Overlap of head length in Pacific mackerel, after Roedel (1952). The small triangle indicates the estimated mean head length at 250 mm. fork length and the hollow bar one standard deviation from regression $s_{y.x}$ either side of the mean, the solid bar two standard errors of the mean $s_{\hat{y}.x}$ on each side of the mean.

should all be lower case letters.

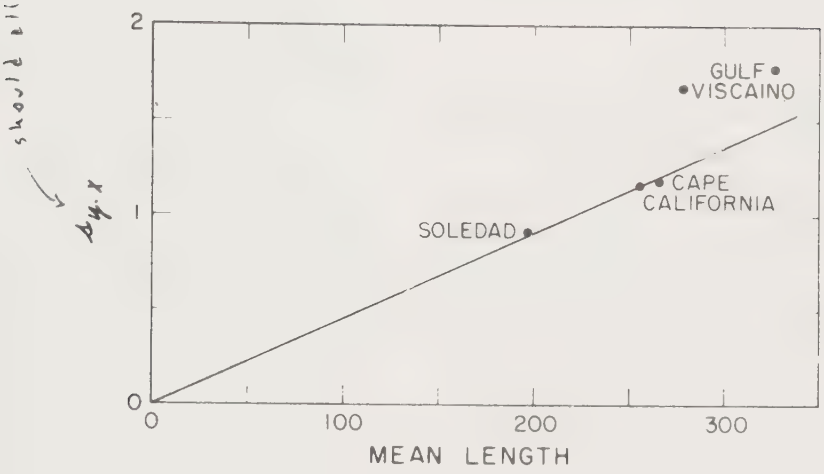

Figure 5.--Relation of mean length to standard deviation from regression in the Pacific mackerel from Roedel (1952).

establish the best separation of the sample.

OVERLAP OF MULTIPLE CHARACTERS

The preceding sections have shown that the amount of overlap between two samples may be computed in the same units for either counted or measured characters. We now consider how to combine the results of comparing several characters either counted, measured, or both in combination.

This is a problem which has long plagued taxonomists. Ginsburg (1939) gives a good review of the methods used by fish taxonomists. These include a great variety of sums, ratios, and products. Usually the combining method has been developed to fit a particular problem and usually, too, the results have been difficult to compare statistically. Then, after a number of characters have been compared, either individually or in some combination, it has been perplexing to interpret the results. Are two correlated characters to be given twice as much weight as one? Are small differences in a number of characters as significant as a large difference in one character? No satisfactory answers have been found by fish taxonomists. Ginsburg concluded that it was best to rely on a "principal" character and place slight weight on other characters.

One elaborate method of combining characters was used considerably by anthropologists and occasionally by other taxonomists, e.g., Royce (1953). This was called a coefficient of racial likeness by Pearson (1926, 1928) who originated and applied it. It is essentially an average of the difference between means divided by the sum of the standard errors of the means, with the result adjusted to samples of a constant size. The adjusted value is known as the reduced coefficient of racial likeness

$$R.C.R.L. = 50\left(\frac{\bar{n}_1 + \bar{n}_2}{\bar{n}_1\ \bar{n}_2}\right)\left[\left\{\frac{1}{M}\sum\frac{\left(m_{s_1} - m_{s_2}\right)^2}{\frac{\sigma_{s_1}}{n_{s_1}} + \frac{\sigma_{s_2}}{n_{s_2}}}\right\} - 1\right] \qquad (11)$$

in which n_s is the mean of the sth character, σ_s the standard error of the sth character, n_s the number of observations of the sth character, $\bar{n}$ the mean number of observations of all characters, and M the number of characters. The subscripts 1 and 2 refer to the first and the second samples respectively. The first parenthesis involving n is the reduction factor, purporting to eliminate the effect of sample size.

The coefficient of racial likeness was severely criticized by Fisher (1936a) and by other statisticians, who generally agreed with Fisher. It has two major deficiencies (Rao 1952). First, and probably most serious, all the characters are treated as though they are independent. The addition of a character highly correlated with one previously considered may produce a large change in the coefficient even though it adds no additional statistical information. Second, even the reduced coefficient is not independent of sample size, which in taxonomic material is rarely uniform. Associated with this is the fact that the weight given various characters depends on the number of measurements rather than on any biological criteria. Fisher (1936a) also pointed out that adding the ratios of mean differences to their standard error was comparable to repeating a test of significance, something which has no sound logical basis. Once a test of significance has been made, the conclusion has been reached and repeating it is mere reiteration. Because of these difficulties the coefficient of racial likeness has fallen into disuse.

Another approach to multiple character analysis has been made by psychologists in their "multiple factor analysis". This is a method of reducing the number of characters by "factoring" to a smaller number of parameters based on the intercorrelations of the characters. According to Thurstone (1947), however, this method of analysis has been challenged by mathematical statisticians and he admits no direct relation to modern statistical theory. A recent user (Stroud 1953) points out that it is a method of obtaining semireliable explanatory information and there are no adequate methods of establishing confidence in the results

At about the same time that he condemned the coefficient of racial likeness, Fisher (1936b) proposed a discriminant function especially for

taxonomic problems. This consists of a value

$$D = \ell_1 d_1 - - - - \ell_i d_i \qquad (12)$$

which is a maximum obtained by varying the coefficients $\ell_1 - - - - \ell_i$ independently while $d_1 - - - - d_i$ are the differences between the means of i characters in two samples. This paper was followed by two others (Fisher 1938, 1940) in which he compared the discriminant function with Hotelling's (1931) generalized test of significance and with Mahanalobis' (1936) generalized distance function and then developed tests of significance for the discriminant function.

The discriminant function has been used occasionally in taxonomic work. Mather and Dobzhansky (1939) were able to use it with morphological characters to distinguish between two races of Drosophila which had been thought to be morphologically identical although physiologically and ecologically distinct. Stone (1947) used it with counted characters in a study of subspeciation in Boleosoma nigrum, a small fish. By means of it he was able to show that there was no overlap between two forms called subspecies and considered that they should be designated as species. These two instances are, however, rather unusual and there has been no widespread use of the method, at least in fish taxonomy. The reasons are probably that the mathematics are complicated and, more important, that the method is essentially a method of assigning individuals to known groups.

A statistic which gets at the heart of the taxonomic problem of determining overlap is the generalized distance function as stated by Mahanalobis (1936). He started with the case of p independent variates in two statistical populations where

$$pD^2 = \sum_1^p \frac{\left(\bar{x}_{i_1} - \bar{x}_{i_2}\right)^2}{s_{ii}^2} \qquad (13)$$

If this is reduced to the case of a single variate, it is important to note that

$$D^2 = \frac{\left(\bar{x}_1 - \bar{x}_2\right)^2}{s^2} \qquad (14)$$

is merely the square of our equation (5) and the D is equivalent to the one which we have used in measuring overlap.

Mahanalobis (1936) then generalized to the case of p correlated variates in two populations

$$pD^2 = \sum_1^p \sum_1^p w^{ij} (\bar{x}_{i_1} - \bar{x}_{i_2}) (\bar{x}_{j_1} - \bar{x}_{j_2}) \qquad (15)$$

in which w^{ij} is the reciprocal of the variance-covariance matrix w_{ij}. Fisher (1938) pointed out that the p was unnecessary and the formula has been reduced to

$$D^2 = \sum\sum w^{ij} d_i d_j \qquad (16)$$

in which d_i and d_j are the differences between the means.

Mahanolobis' approach to the problem of determining the distance between populations was essentially intuitive, but Rao (1947) supplied a logical solution in which he defined the distance between multivariate populations in terms of the overlapping and pointed out that this distance is an explicit function of D^2. Rao (1947, 1952) also points out that D^2 satisfies two fundamental postulates of distance:

1. The distance between two groups is not less than zero.

2. The sum of distances of a group from two other groups is not less than the distance between the two other groups (triangle law of distance).

A further empirical requirement is also satisfied:

3. The distance must not decrease when additional characters are considered.

This generalized distance function has been applied to taxonomic problems largely by members of the Indian school of statistics. The most extensive use was probably that of Mahanalobis et al. (1949), who made a monumental anthropometric study of over 2,000 individuals in 22 groups using 12 measurements. Several other examples as well as a thorough mathematical treatment are given in the text by Rao (1952).

The method of computation using formula (15) involves a matrix inversion and Rao (1952) suggests that this is suitable for up to about 4

characters. For more he recommends an alternate which makes the nature of D^2 apparent to those not acquainted with matrix analysis. He starts with the pooled estimates of the intragroup correlations λ_{ij} and standard deviations. Then he constructs a table of the normalized mean values $x_1 - - - x_p$ for the p characters in each group--in other words the difference of each mean from the grand mean for the character divided by the intragroup standard deviation. The normalized mean values are then transformed to values $Y_1 - - - Y_p$, which are uncorrelated, and subsequently to other values $y_1 - - - y_p$, which have unit standard deviation. The general formulas are

$$Y_p = x_p - a_{pp-1} --- \ldots --- a_{p1} Y_1 \qquad (17)$$

$$a_{ij} = \frac{b_{ij}}{V(Y_j)} \quad j < i-1 \qquad (18)$$

$$b_{ij} = \lambda_{ij} - \sum_{t=j-1}^{1} a_{jt} b_{it} \qquad (19)$$

$$V(Y_i) = \lambda_{ii} - \sum_{j=1}^{i-1} a_{ij} b_{ij} \qquad (20)$$

$$y_i = \frac{Y_i}{\sqrt{V(Y_i)}} \qquad (21)$$

The values a_{ij} and b_{ij} are convenient intermediate steps in the computations, $V(Y_i)$ is the variance of Y_i, and y_i is the final transformed value of the normalized mean.

The meaning of the computations is more readily apparent from the simplified formulae for the first two transformed means. The first is

$$y_1 = Y_1 = x_1 \qquad (22)$$

or no transformation at all. Then the generalized distance D between two samples using the first character is merely the difference between the normalized means. The second is

$$y_2 \sqrt{V(Y_2)} = Y_2 = x_2 - \lambda_{21} Y_1 \qquad (23)$$

or the second character is reduced by the amount of correlation with the first and then adjusted to unit standard deviation by dividing by the square root of the variance (the standard deviation). The subsequent formulae become much more complicated because of the need for accounting for all of the intracharacter correlations. The reader is referred to Rao (1952) and Mahanalobis et al. (1949) for a complete explanation.

The applications of the generalized distance have all concerned studies which used counted characters or measured characters considered to be independent of total size. In other words, no regression was involved. In our tuna studies, where regression is involved, we substitute the estimated mean length at a given size for the mean, the standard deviation from regression for the standard deviation, and the intragroup partial correlation coefficient (independent of total length) for the simple correlation. The multivariate analysis of tuna populations will be the subject of ensuing papers.

THE SAMPLING PROBLEMS

Before we turn to a comparison of morphological and tagging studies it is necessary to discuss the sampling problems. No matter how good our statistical treatment of the data, the inferences which we draw can be no better than the sampling. Here is a problem of special difficulty not always carefully considered by those concerned with tuna morphometric data. Most of our discussion is based on the recent treatment of sampling by Cochran (1953) and Cochran et al. (1954).

A good sample must be a random one or some modification which does not change the basic principle that every individual in the population has an equal or known chance of selection A random sample is a mathematically precise concept. Its importance is becoming widely recognized because not quite random samples are found to be unreliable.

If we are to obtain precisely a random sample we must first accurately specify the population which is to be sampled. In the usual taxonomic study this cannot be done for the biological population because the limits are not known and the purpose of the study is frequently to describe them. We can, however, call the biological populations

the target populations and recognize sampled populations which have defined boundaries in space and time. From the characteristics of the sampled populations we must infer the nature and the bounds of the target populations. Ginsburg (1938) recognized the sampling problem and emphasized the need for obtaining a sample from as many locations as possible and for avoiding excess representation from any locality. He called his complete sample a composite which was made up of constituent samples.

After specifying the bounds of the sampled population, the fish taxonomist must further specify the method of sampling, because fish vary greatly in their availability to all kinds of gear, especially at different sizes and even within a size group of a species. Inevitably the nature of fishing gear makes the catch a non-representative sample. Almost always a catch consists of members of a school, or of an aggregate, which more closely resemble one another than they do the balance of the sampled population. Frequently, too, the location of the catch cannot be randomized in the specified area or time because of economic factors. Therefore the individuals within catches should be expected to resemble one another more than in a random sample. It follows that estimates of variability from the usual samples will tend to be too low. Estimates of the difference between means of such samples in terms of either overlap or significance of the difference will tend to be too high.

Once we depart from a random sample we forsake exactness and can only say vaguely that the results must be treated with caution. Therefore in any discussion of morphometric data the sample characteristics should be specified in detail.

EVALUATION OF INTERMINGLING FROM MORPHOLOGICAL STUDIES AND TAG RETURNS

In all of the recent morphometric studies of tunas the conclusion has been reached that statistically highly significant differences exist between the samples being compared. The odds against the null hypothesis have been such that there is no doubt that the samples were not drawn from a single completely mixed population. There has been a tendency to conclude from this that the populations are distinct.

These studies have not determined the proportion of individuals within the groups that are identical on the basis of the measurements used. It must be recognized that this proportion of individuals might have come from the same parent stock. Of course, there is no proof that they did, but then the data provide no proof that they did not. It follows that the proportion with identical characteristics is a maximum proportion which might have come from the same parent stock (intermingled) and that the true proportion is equal or lower.

Two recent authors have had an opportunity to compare morphological studies with tagging results. Both of them found statistically highly significant differences in morphology between populations which tagging studies showed to be intermingling to a considerable degree. One of these authors concluded that such results were contradictory but we believe that such results are not necessarily contradictory and we propose to examine them here in detail.

In one comparison of morphological studies and tag results Tester (1949) used the vertebral counts of large numbers of herring taken in five areas off the west coast of Vancouver Island. He found statistically highly significant differences in the number of vertebrae among all areas, but the differences in mean vertebral number were very small. We have computed the overlap between the two most widely separated areas which showed the greatest differences in mean number of vertebrae (pages 15-16, fig. 3), and even here the overlap is almost complete, $p = .465$, $\mathcal{S}\Omega = 93\%$. Considering this, the findings from the tagging studies, which indicated that about 45 percent of the fish tagged in one area wandered to other areas, are not surprising.

In the second example Roedel (1952) compared samples of Pacific mackerel from five areas between southern California and the Gulf of California. He found statistically highly significant differences in certain vertebral characters and in head length and postulated that there were

five reasonably distinct populations, among which little mingling would be expected. He states that this conclusion was not compatible with evidence from the tagging experiments (Fry and Roedel 1949:25), which showed substantial amounts of intermingling between southern California and the Soledad and Viscaino regions off Baja California. Of the fish released off Soledad 6.8 percent were recovered from southern California, and of those released in the Viscaino area 2.4 percent were recovered from southern California. These returns may be compared with the overlap of head length (p. 17), which for the California-Soledad comparison was 90 percent and for the California-Viscaino comparison was 69 percent. In both comparisons it is evident that a major part of the individuals possessed identical head length characteristics.

There is also reason to suspect that the samples were not adequately representing the mackerel populations throughout the year in the areas specified. The standard errors of estimate were not uniform among regions (p. 19); the Viscaino and Gulf regions showing unduly high values suggestive of a more heterogeneous population. The high value for the Gulf results from only two samples taken on successive days in February 1941, whereas the samples for the Viscaino region resulted from six subsamples, most of which were taken on separate days. Again, Soledad was represented by only two subsamples and California by only five subsamples. Some further evidence of heterogeneity is apparent, because the regression coefficient of head length on fork length is different between California-Soledad, Viscaino-Cape, and Cape-Gulf. These differences are such that the small fish showed little difference between the Cape and the Gulf and between California and Soledad and the large fish between Viscaino and the Cape. Roedel further notes that the population in the Viscaino area showed marked differences between the 1941 juveniles and older fish with respect to vertebral characters.

The samples that were tagged may have been even less representative, because almost all of those released in the Soledad area were tagged in January 1940, with a few in October 1941. In the Viscaino area about 80 percent were released in February 1941 and in October 1941, with the balance scattered among several other months. If, as the above evidence indicates, this mackerel population is heterogeneous, with groups moving seasonally, and perhaps independently, north or south along the coast, it is obvious that fish sampled for either tagging or morphometric studies at one or two times during the season may not be representative of the population in the area during the entire season.

Therefore it is not warranted to conclude, as Roedel did, that the morphological studies are not compatible with the evidence from tagging. The morphological studies showed that the stocks within an area were heterogenous but that even between areas a large proportion of the individuals had identical characteristics. Neither morphological nor tagging studies were based on samples reasonably representative of any area nor even of any definite period in any area, so it is not possible to estimate the amount of intermingling. The tagging studies show there was some intermingling, the morphological studies that the intermingling was not complete; there the matter must rest.

Thus tagging and morphometric studies may provide essentially opposite and mutually supplementary estimates of intermingling. A tagged fish released in population A and returned from B shows that intermingling has occurred. If the sampling in both populations A and B has been adequate and the tagged fish are representative of population A, then the proportion returned from B will be an estimate of the intermingling of A with B. Such conditions rarely can be satisfied in practice and hence we must usually stop with the estimate that some intermingling occurs. On the other hand a maximum amount (but no proof of occurrence) may be fixed by the amount of overlap of morphometric characters. The two methods may then enable us to make a statement to the effect that intermingling occurs but does not exceed a certain amount.

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MEASUREMENT OF POPULATION MOVEMENT BY OBSERVATION OF MERISTIC OR MORPHOMETRIC CHARACTERS

By

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INTRODUCTION

When the general appearance of the individual fish in the catch in a particular region suddenly changes in a short period of time, clearly the populations fished have changed composition. That is, new, different-looking fish have moved into the region from somewhere outside it, during the time that the change in appearance took place. By measuring the characters in which change is observed, it is possible to state, under certain conditions and within sampling limits, what percentage of the changed population came from elsewhere. (The populations referred to will be defined ordinarily as those fish in a given area at a given time; they are not necessarily genetically specified populations.)

Tag return data yield a complementary measure of movement; the percentage of the population in one region that moved to another region. Both techniques depend upon a certain lapse of time between observations, and the percentage amounts in both cases are rates of movement or exchange. They can therefore be expected to vary with the length of the time interval, and also at different time periods.

In brief, we wish to propose a method whereby the amount of mixing of two different fish populations can be measured. (It is important to note that no judgment is made about the nature, genotypic or phenotypic, of the differences observed. This is a separate problem whose solution depends on a different kind of information.) We will give a hypothetical example and discuss the general application of the method, including sampling and confidence limits. An actual example will be drawn from Pacific sardine data. Finally, the benefits of combining this method with a tagging experiment are mentioned.

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A SIMPLIFIED HYPOTHETICAL EXAMPLE

Let there be two adjacent regions inhabited by a certain species of fish of a single year-class. Let the mean value of a certain meristic character of the fish be different in the two areas at a given time. Let the distribution of this character in these two populations overlap considerably.

Now let a certain number of fish in Region A move into Region B within a certain time, say a year. The mean value of the character in question will not have changed in Region A, but it will have changed in Region B; it will have shifted toward the mean value in Region A. The amount of this shift is completely dependent upon two things: First, the percentage of fish now (in the second year) in Region B that came from Region A, and, second, the amount of the difference in means in the first year between the two regions. To simplify the expression of this situation, let

X_{ali} be the value of the character for the ith fish in Region A in the first year,

M_{al} be the mean in Region A of the character in the first year

X_{bli} be the value in Region B of the character in the first year

M_{bl} be the mean in Region B of the character in the first year

X_{b2i} be the value in Region B of the character in the second year

M_{b2} be the mean in Region B of the character in the second year.

Let there be

N_1 fish in Region B in the second year that were in Region A in the first year

N_2 fish in Region B in the second year that were in Region B in the first year.

The percentage of fish in Region B in the second year that came from Region A since the first year, then, is P in

$$(1) \qquad P = \frac{N_1}{N_1 + N_2}(100)$$

The sum over all fish of the value for the character in question in Region B in the second year is

$$\sum_{i=1}^{N_1 + N_2} X_{b2i} = (N_1 + N_2)M_{b2}$$

and is also equivalent to

$$(2) \qquad (N_1 + N_2)M_{b2} = N_1M_{a1} + N_2M_{b1}$$

Rearranging the expression (2) above, one finds that

$$(3) \qquad P = \frac{M_{b2} - M_{b1}}{M_{a1} - M_{b1}}(100)$$

So if the three means are known (the mean of fish in Region B in the second year, of fish in Region B in the first year, and of fish in Region A in the first year), the percentage of fish in the second year in Region B that came from Region A since the first year is uniquely determined.

Two conditions are necessary to the above conclusions:

1. The means M_a and M_b do not change with time and,

2. The fish that move, N_1 in number, must have the same mean as those remaining in Region A, namely M_{a1}

These two conditions are probably satisfied in the case of meristic characters of adult fish, but probably are not for many morphometric characters. One morphometric character that normally would satisfy them, however, is the "calculated" length of a fish at some given age. An example given later will be based on such data.

GENERAL APPLICATION;SAMPLING AND CONFIDENCE LIMITS

Rarely will a situation as simple as the example given above be found in practice. Usually the biggest problem will be to describe the means of populations in all adjacent areas inhabited by the fish in question. The difficulty then will be one of deciding from which region the movement originated. A change in the mean between time periods at a given region proves that some fish have entered that region. To find out what proportion are immigrants, one must know the mean value of those that entered.

If adjacent regions contained fish previously that could, by immigrating, also have altered the observed mean, then the amounts of each, taken one at a time, necessary to have accomplished the change, can be computed. It remains a matter of judgment from other evidence as to which region or regions affected the local mean.

In practice, the true mean value of a character is never known, since only a part of the population is observed. The mean of the samples will approach the true mean to the degree that the samples are representative of the population. If the definition of populations is restricted to only those regions that are uniformly or representatively sampled, apprehension and bias will be avoided. Within representatively sampled areas, larger samples will be more accurate in describing the population. If some areas known to contain fish are not sampled representatively, it is better to restrict conclusions to only those that are sampled representatively, rather than to try to extend them to all areas, sampled or not. While the former results will be limited, the reason will be incomplete coverage and not improper sampling. The areas not sampled will then stand out as those in which future work should be done.

The distribution of most characters will not be normal, but will be skewed with a definite

maximum. For the degree of skewness usually encountered, the distribution of sample means from such frequency distributions can be presumed normal. The variance of the mean will be the variance of the characters' frequency distribution, divided by the sample size.

Similarly, the distribution of the difference between such means will be normal with variance equal to the sum of the variances of the two means whose difference is considered.

The distribution[1/] of the sample estimate of "P", say $\hat{P}$, will generally not by symmetrical. If

$$\hat{P} = \frac{m_{b2} - m_{b1}}{m_{a1} - m_{b1}} = \frac{\bar{y}}{\bar{x}}$$

where the m's are the sample means, the α % confidence limits for $\hat{P}$ are

$$\hat{P}\left[\frac{1 \pm t_{n,\alpha}\sqrt{C_{\bar{x}}^2 + C_{\bar{y}}^2 - t_{n,\alpha}^2 C_{\bar{x}}^2 C_{\bar{y}}^2}}{1 - t_{n,\alpha}^2 C_{\bar{x}}^2}\right]$$

where

$$C_{\bar{y}}^2 = \frac{\sigma^2(\bar{y})}{\bar{y}^2} = \frac{\sigma^2(m_{b2}) + \sigma^2(m_{b1})}{(m_{b2} - m_{b1})^2}$$

and

$$C_{\bar{x}}^2 = \frac{\sigma^2(\bar{x})}{\bar{x}^2} = \frac{\sigma^2(m_{a1}) + \sigma^2(m_{b1})}{(m_{a1} - m_{b1})^2}$$

and t_α is the "α" level of the normal or "Student" distribution. These roots may exceed unity or may be negative, and may even be imaginary, but only when the coefficients of variation are large. For coefficients of variation of y and x greater than 0.7 the roots will often be imaginary. Although one might be tempted to conclude in such cases that one knows less than nothing about the reality of P, they will usually be interpreted as simply inadequate sample size.

AN EXAMPLE FROM THE PACIFIC SARDINE (SARDINOPS CAERULEA) FISHERY

There are a number of different kinds of data available from the sardine fishery which could serve to illustrate the method. Among these are calculated lengths of fish at earlier ages, based on the proportionality of scale growth and fish growth. Such characters should be constant throughout life for a given individual, satisfying the conditions stated above.

We have selected the calculated length at the end of the first year of life of fish of the 1945-class taken in the 1947-48 and 1948-49 seasons in the San Pedro and Monterey fisheries. The frequency distributions of these calculated "l_1's" are given in table 1. The means, their estimated variances and other statistics are given in table 2. The 1945 year class is being used as an example. Each year class could be similarly treated.

As may be noted from table 2, the mean l_1 of the 1945-class in San Pedro in the 1947-48 season was 138.1 mm., some 37.5 mm. greater than the comparable mean at Monterey. In the following (1948-49) season, the mean l_1 of the 1945-class at San Pedro had changed to 129.9 mm., or 8.2 mm. less than it had been in the previous season.

If an influx of fish from Monterey were responsible for the decreased San Pedro average, one can estimate what portion of the San Pedro 1948-49 landings must have been fish from Monterey of the past year. This portion can be estimated as the ratio of the change in mean at San Pedro to the difference between San Pedro and Monterey means in the "first" year, 1947-48. This would be the ratio of 8.2 to 37.5, or 0.2187, approximately 22 percent. In other words, if 22 percent of the San Pedro samples in 1948-49 were fish from Monterey in 1947-48, the San Pedro mean would have changed from 138.1 mm. to 129.9 mm.

Referring now not to the samples, but to the population from which they were taken, some allowance for sampling variation must be made. A 95 percent confidence interval for the ratio .2187 in this example would be .328 and .1121, or approximately 11 to 33 percent. The computation of these limits is outlined in table 2.

1/ Cochran, W. G. "Sampling Techniques," New York, John Wiley and Sons (1953), see p. 121.

DISCUSSION

The condition that the only fish new to San Pedro were those from Monterey should be examined. The next port of landing south of San Pedro is Ensenada, Mexico; however, no samples are available for the season 1947-48. If there had been samples, one should have examined the "l_1" distribution of the 1945 class, since if the mean l_1 there happened to be also less than that at San Pedro, the change at San Pedro between the two seasons could alternatively have been due to an influx of fish from the south, as well as from the north. No unique solution would exist in such a situation, but if one considered the fish moving only from the south, the portion necessary to alter the San Pedro mean as much as observed could be calculated in the same fashion as has been done here for Monterey. Similarly, offshore regions, if they contained fish whose l_1 was less than that at San Pedro, could be responsible for the decreased San Pedro mean. If the mean in all outlying regions but one were greater, the one region would necessarily be responsible.

Other combinations of mixing from other regions are possible: the technique above requires the condition that one region at a time be considered.

TECHNIQUES COMBINED WITH TAGGING

If the populations are tagged, the above study yields useful complementary measures of population movement. The tagged fish, when recovered, tell one what portion of an outside area's population moves into a given area. The change in the mean of the fish's attribute tells one what portion of the given region's population came from a given outside area. The ratio of the population size in the tagged area to that in the untagged but recovered area can then be estimated. If P_1 is the proportion of fish in the recovery area that came from the tagged area and P_2 is the proportion of fish of the tagged area that moved from the tagged area into the recovery area, then the relative population size is $N_1/N_2 = P_2/P_1$. If, further, the size of the population in the tagged area is known (from tagging and recovering there), the population size in the non-tagged area can be estimated. The change in means would be referred to the locality of tagging and that to which tagged fish moved, in computing the movement on the basis of fish attributes. The movement of the tagged fish provides a basis for deciding where the immigrant fish came from that produced the observed change in fish attributes' mean. Since the analysis of time changes in the mean value of fish attributes can yield estimates of population movement only if assumptions are made about the direction of movement, the method is far more powerful if tagging data for adjacent regions are available.

SUMMARY

One of the persistent problems of fishery biology has been the determination of movement of fish within a population occupying a given geographical area. We have proposed a method for estimation of the amount of interchange between sub-areas based on the time differences in mean values of attributes of the fish. Since mixing is a dynamic process, conclusions about its magnitude must be based upon observations taken in succeeding time periods.

The true difference in an attribute's mean between time periods of a region subject to migration from an adjacent area depends on both the difference in means of the mixing fish and the extent of the migration into the region. Providing first that the migrating fish are representative of the fish in the adjacent area, second, that the attribute is constant with the passage of time, and third, that the change in mean between years may be solely attributed to mixing from a given adjacent region, then a simple method of estimating the magnitude of mixing has been presented. Confidence limits for an estimate of the proportion of fish in the local region that have moved from the adjacent region have been written out. It is important to remember that this technique will not yield an estimate unless real differences in the two regions' means are established. For this reason consideration should be given to the number of fish sampled from the regions, and they should be taken in such a manner that the samples are representative of the fish populations.

The main difficulty in application of the method lies in establishing the source of immigrants, and the usefulness of tagging data in this regard has been described.

Table 1.--1945 year class, calculated length at one year of age

Length mm. class midpoint L	1947-48 season		1948-49 season
	Monterey frequency f	San Pedro frequency f	San Pedro frequency f
68.5	-	-	1
72.5	-	-	0
76.5	5	-	0
80.5	2	1	0
84.5	5	0	2
88.5	7	1	1
92.5	13	1	2
96.5	14	4	1
100.5	10	1	4
104.5	7	2	2
108.5	11	1	1
112.5	12	4	3
116.5	7	5	2
120.5	1	8	2
124.5	5	12	10
128.5	1	26	12
132.5	-	34	9
136.5	-	27	12
140.5	-	36	10
144.5	-	32	10
148.5	-	39	7
152.5	-	15	5
156.5	-	20	0
160.5	-	5	0
164.5	-	4	2
168.5	-	3	1
Total	100	281	99
ΣfL	10,106	38,944	12,906
ΣfL^2	1,036,705	5,453,546	1,715,505

Table 2.--Outline of computation of percentage movement and its confidence interval. (See text for explanation.)

Monterey		San Pedro	
1947-48	$\bar{L}$ = 100.6 mm.	1947-48	$\bar{L}$ = 138.1 mm.
	N = 100		N = 281
	s_L^2 = 155.5 mm.^{2}		s_L^2 = 200.5 mm.^{2}
	$s^2(\bar{L})$ = 1.555 mm.^{2}		$s^2(\bar{L})$ = 0.7135 mm.^{2}
		1948-49	$\bar{L}$ = 129.9 mm.
			N = 99
			s_L^2 = 338.4 mm.^{2}
			$s^2(\bar{L})$ = 3.418 mm.^{2}

$\bar{y}$ = (130.4 - 138.6) = -8.2 mm. . . . . .change at San Pedro

$\bar{x}$ = (100.1 - 138.6) = -37.5 mm. . . . . .immigrant difference

$P = \frac{\bar{y}}{\bar{x}} = .22$

$C_y^2 = \frac{4.132}{(8.2)^2} = 0.061$ $\qquad$ $C_x^2 = \frac{2.269}{(37.5)^2} = 0.00161$

$$P'_u = \hat{P}\left[\frac{1 + 1.96\sqrt{.061 + .00161 - 3.84\,(.061)(.00161)}}{1-3.84(.00161)}\right] = 0.326$$

$$P'_l = \hat{P}\left[\frac{1-1.96\sqrt{.061 + .00161-3.84(.061)(.00161)}}{1-3.84(.00161)}\right] = 0.117$$

Upper 95% limit ($100\,P'_u$) 33%

Point estimate ($100\,\hat{P}$) 22%

Lower 95% limit ($100\,P'_l$) 12%

A REVIEW OF PAPER CHROMATOGRAPHY AS USED IN SYSTEMATICS

By

David A. Farris[1/]

DESCRIPTION OF THE TECHNIQUE

Chromatography, a technique for separating the components of a complex mixture, is based on the differential adsorption and solvation of the components. It was first described by Tswett (1906) and subsequently adapted to paper by Consden, Gordon and Martin (1944). A small quantity of the mixture to be separated, in this case either body fluid or tissue extracts, is placed on a spot not over a quarter of an inch in diameter near one edge of a filter paper sheet. After the spot is dry, the edge of the filter paper is dipped into a solvent so that the solvent flows over the spot by capillarity. As the solvent front progresses over the paper, the components of the mixture are spread out behind the front in discrete areas. To stop the process, one merely removes the solvent and allows the paper to dry. By the use of indicator solutions, ultra violet light, etc., the spots are located for identification. The compounds can be removed from the filter paper by elution and the amounts determined quantitatively. For a more complete description of the technique see Stein and Moore (1951); Strain (1942); Lederer and Lederer (1953); Block, Durrum, and Zweig (1955); and Cramer (1953).

RATIONALE OF APPROACH

The continuity-of-life hypothesis has many corollaries among which are the hypotheses of the "continuity of form" and the "continuity of biochemical systems". The latter hypothesis may be verified by showing the simultaneous occurrence of compounds in various groups of organisms thought, on the basis of other evidence, to be related. It is hoped that by understanding chemical relationships, it will be possible to deduce systematic relationships in cases where the evidence from other disciplines is not sufficiently strong to establish the phylogenic rank of the population under question.

Chromatography can be used for the study of speciation in at least two ways. The investigator may simply examine the qualitative chromatographic patterns of chemical differences existing between populations. In this kind of analysis the compounds are neither identified nor the concentrations determined. This increases the difficulty of comparing the results of independent investigators. No attempt is made to relate the pattern with the phylogeny of the organism and one is left in doubt as to whether pattern differences have any systematic significance. On the other hand, quantitative methods utilizing photometry, etc., have the advantage of permitting a determination of individual as well as species variability.

The lack of understanding of the "species concept" has hampered both the study of evolution and species description. The reader is referred to Stebbins (1950), Mayr (1942), Simpson (1953), and Myers (1952) for a discussion of the mechanisms of speciation. Populations normally referred to as different species (sometimes) differ from each other with respect to spatial and temporal separations, and the kinds and numbers of genetic differences. Basically, the study of speciation may be restated as a study of the change in gene pools brought about by changes in the individual germ plasms within the pools and selection of those changes under the influence of chance and environment.

An examination of populations with respect to morphological manifestations of genetic differences although usually fruitful is subject to some error because of the environmental effects on gene expression. Ideally, an

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examination of the genes themselves would circumvent this difficulty. Although it is impossible to do this at present it is possible to examine differences in the presumed primary gene products, the enzymes controlling specific reactions. The assumption made here is that the greater the number of enzyme systems held in common by different populations, the closer their phylogenic relationship.

APPLICATION OF CHROMATOGRAPHY

Literature in this new field of systematic chromatography is rather sparse but surprisingly diversified. Rather than present the work in chronological order, I have chosen to take it up phylogenically.

Three groups of plants have been investigated. Buzzati-Traverso (1953) chromatographed varieties of both the tomato, Solanum lycopersicum L., and the muskmelon, Cucumis melo L. Selle (1954 a, b) worked with varieties of the sour orange, Citrus aurantium L. The tissues used were pollen, root tips, leaf extracts, and root bark. The compounds examined were ninhydrin positive and fluorescing materials. In all cases only the chromatographic pattern was examined, but this was sufficient to separate the varieties under scrutiny

Among the animals, seven species of land snails were investigated by Kirk, Main, and Beyer (1954). The chromatographic patterns of ninhydrin positive and fluorescing compounds extracted from foot muscle tissue were examined and the seven species could be distinguished. Four of the species were of the same genus. Three of these species had strikingly similar patterns.

Utilizing essentially the same technique, Hadorn and Mitchell (1951) and Buzzati-Traverso (1953) chromatographed the bodies of Drosophila melonogaster, and were able to distinguish various mutants. In some cases the heterozygotes could be distinguished from the homozygotes. Changes in the pattern were also observed during metamorphosis.

Culicid mosquitoes were examined for free amino acids by Clark and Ball (1951, 1952), Ball and Clark (1953) and by Micks and Ellis (1952). The several species had distinct amino acid patterns which were fairly constant irrespective of the area in which the mosquitoes were collected. At the same time it was shown that there was a change in the free amino acid complement which accompanied metamorphosis.

Williams (1951) and coworkers have been using the body fluids of humans for chromatographic analysis and have quantitatively analyzed a wide variety of compounds. The object is the biochemical characterization of both the population and subpopulation. In this case, a suggested subpopulation is composed of alcoholics having a unique population of compounds which may be associated with alcoholism. Once the association is made, it will be necessary to show whether an array of compounds is associated with the cause of alcoholisms (genetic) or whether the array is the result of alcoholism (environmental).

Dannevig (1955), working with fish, has shown that some 23 species of fish have different ninhydrin-positive compound patterns. Likewise, Buzzati-Traverso and Rechnitzer (1953) have shown that various species of fish can be separated on the basis of their chromatographic patterns. They state that the closer the two species are phylogenetically, the more similar are the chromatographic patterns. Farris[1/] made a study of the Pacific sardine, Sardinops caerulea, with respect to environmentally induced variation in the chromatographic pattern, and found that the pattern could be changed quite significantly with respect to at least two components of the free amino acid complex. This change was correlated with the relative fatness and with the diet of the fish.

DISCUSSION

It is apparent that this work is still in an exploratory phase. A rigorous evaluation of the method is very difficult because of the paucity of data. On theoretical ground, systematic chromatography holds great promise. The amount of material is practically unlimited.

1/ Ms. "Diet induced variation in the free amino acid complex of Sardinops caerulea".

There are about 4×10^{26} possible combinations of the 26 naturally occurring amino acids. Additional compounds allow combinations far in excess of the number of known or anticipated species.

From the preceding resumé of the literature, it appears that closely related members of a genus can be distinguished on the basis of their chromatographic patterns. That is to say, the biochemical data tend to be consistent with the morphological data and with some genetic data. However, before the results of this technique can be used as prima facia evidence for speciation, the limits of individual variation will have to be determined.

Ross, Holtman, and Gilfillin (1955) have shown that a bacterial infection in chicks materially reduces the amounts of four amino acids in blood and liver tissue. The variation imposed by diet and age has been demonstrated. Other sources of organismal variability, such as sex, and environmental variability, such as temperature, should be evaluated.

As with classical systematics, the investigator will have to know something of the intra-generic biochemical variation before he can make any statements about the significance of intra-specific variation. Although many of the same difficulties met at the specific level will be encountered at the sub-level, the magnitude of variability will be reduced and therefore harder to assess. Differences between species are usually so great that they can be recognized at the morphological level, but the more subtle changes in the genome associated with subspecies may be hidden. The resolving power of chromatography at these low levels of variation may prove to be most useful in the separation of such populations. Just as morphology has been useful for the study of speciation, comparative biochemistry may become a powerful tool for investigation of subspeciation.

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THE USE OF IMMUNOLOGICAL TECHNIQUES IN RACIAL STUDIES

By

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Races have been defined as populations which are characterized by different frequencies of variable genes and/or chromosome structures (Dobzhansky and Epling, 1944). There is much evidence from the work of geneticists, systematists and physical anthropologists to support this definition. It would appear then that one of the best ways to distinguish between races of any animal would be to find variable characters which are genetically controlled and determine their frequency within the populations in question. Gordon (1947) has used this method in a study of isolated populations of the Mexican platyfish, (Platypoecilus maculatus). The characters he studied, seven different patterns of tail markings, were shown to be due to a series of dominant allelic genes. Populations from different geographic areas were shown to have different and characteristic frequencies for these genes, some of which have remained fairly constant over a period of 70 years.

The characters which have been found most useful in studies on the races of man are differences in the antigenic composition of red cells. The first such differences, now known as the ABO groups, were discovered by Landsteiner (1900), shown to be genetically controlled by Von Dungern and Hirszfeld (1910) and first demonstrated to differ in their frequency of occurrence in various races by L. and H. Hirszfeld (1919). Subsequently, many other antigenic differences in human red cells have been found and demonstrated to vary in frequency between various populations or races. For recent reviews concerning this subject, see Boyd (1950), Race and Sanger (1954), Mourant (1954) and Levine (1954).

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Individual differences in red cell antigens have been demonstrated in many animals including the following: cattle (Stormont, Owen and Irwin, 1951), sheep (Ycas, 1949), chickens (Briles, McGibbon and Irwin, 1950), whales (Fujino, 1953) and goldfish (Hildemann, 1954). These antigenic differences have been shown to be genetically controlled in cattle and in chickens wherever they have been studied by genetic techniques. Differences in the frequencies of cellular antigens between breeds (races) have been shown in dairy cattle (Owen, Stormont and Irwin, 1947). There is also some evidence that isolated stocks of sheep (Dujarric de la Rivière et al., 1952) and of whales (Fujino, 1953) differ in the frequency of occurrence of various cellular antigens.

Another serological property which shows variation between individuals is the presence of naturally occurring hemagglutinins or antibodies which agglutinate red blood cells. These invariably occur in man in a reciprocal relationship to the A and B antigens and with them define the ABO blood groups. Of interest here are the demonstrations of individual variations in the occurrence of natural hemagglutinins in yellowfin tuna and skipjack bloods (Cushing, 1952) and the bloods of several species of whales (Fujino, 1953). They have been tested for, but have not been found in the blood of the cod, (Gadus morrhua) (Jensen, 1937). The usefulness of natural hemagglutinins for racial studies is limited by the fact that they occur irregularly except in man and there is considerable doubt that they are genetically controlled (Wiener, 1951).

The antigenic properties of serum proteins have been extensively used to demonstrate phylogenetic relationships between animal species (Nutall, 1904), including fish (Gemeroy, 1943). However, intraspecific differences in the antigenic properties of serum proteins have been demonstrated only once to the author's knowledge

(Cumley and Irwin, 1943) and have not been used in racial studies. It would therefore seem that antigenic differences in serum proteins offer little promise for racial analysis in fish. Nevertheless, the proper application of the new serological techniques known as agar diffusion may demonstrate useful intraspecific differences. Serum proteins have one advantage over red cells in that they are easily preserved by freezing and can be held for much longer periods of time.

From the work done on other animals and man, it seems that the immunological techniques most likely to yield results which can be used in racial analyses in fish are those used to demonstrate intraspecific differences in cellular antigens. These are illustrated in figure 1 and consist of giving experimental animals a course of injections with washed blood cells from individual fish of the species being investigated. The surface of these red cells contains high-molecular-weight substances which are foreign to the experimental animal and consequently serve as antigens, i.e., they cause the animal to respond by producing specific antibodies against them. This process is identical to vaccination. As we all know, vaccination against typhoid does not make us immune to diphtheria; this is because the antibodies produced are so specific that they will react only with the antigen which induced their production or with very closely related antigens. In the illustration, the different surface antigens are characterized diagrammatically as different geometric shapes, some of which vary between the cells of the two individual fish and some of which are present on the cells of all members of the species. The specificity of the antibodies formed is illustrated by indentations in them so that they fit their corresponding antigens much as a hand fits a glove or a key fits a lock. In order to make the antisera thus produced useful in differentiating between fish of the two types, we must remove all the antibodies to the antigens which are common to the two types. This is accomplished by the technique of absorption. The antisera to type FA is mixed with red cells of type FB and correspondingly the antisera to type FB is mixed with red cells of type FA. When this process is properly carried out, all the antibodies to common antigens are removed when the red cells are centrifuged down. Only type specific antibodies remain in the serum - that is, they will agglutinate only red cells which possess the antigen which characterizes one antigenic type. These antibodies are not removed because they do not fit any of the antigens present on the red cells used for absorption. Sera which are prepared in this manner can be used to determine the antigenic types of individuals in populations of interest and to determine the frequency of occurrence of these types. If statistically significant differences in these frequencies are found, we have tools for studying the geographic range, extent of straying, precision of homing and other characteristics of the populations in question. Of course, the greater the number of antigenic characteristics which we have sera to test for, the more likely we are to find differences in frequencies between populations of interest.

The illustration shows rabbits as the experimental animal used for antibody production. However, one should use other animals as well, preferably animals as closely related to the donor as possible. The ideal procedure, and one which has been used to demonstrate many individual antigenic differences in man, cattle, sheep, chickens and goldfish, is isoimmunization. This consists of using members of the same species for both donor and recipient. Antigens possessed by the donor and not the recipient are foreign to the latter and antibodies will be produced to these substances just as they would be to any other foreign antigen. Finer antigenic differences are usually demonstrable by isoimmunization and, of course, antibodies against species-specific antigens are not formed so they need not be removed by absorption. When using fish as an antibody former, one must keep in mind the fact that their rate of antibody production is dependent on the temperature of the water in which they are held (Snieszko et al., 1938) (Cushing, 1942).

One of the biggest technical problems during developmental work to discover red cell antigens is the necessity for having fresh red cells for testing the antisera and for absorptions. In the case of fish, especially pelagic and anadromous fish, it is not always possible to obtain blood samples repeatedly from the same individual. Two methods of preserving red cells which were

originally developed for mammalian cells show some promise of applicability to fish. The first method, holding blood at 4°C. in the presence of a small amount of isotonic acid-citrate-dextrose solution has been shown to be applicable to goldfish cells (Hildemann, 1954). We have found this method works quite well with the cells of chinook salmon, (Oncorhynchus tshawytscha), but is not as successful with the cells of sockeye salmon, (Oncorhynchus nerka). The second method, freezing the cells in the presence of glycerol, has been applied to the erythrocytes of the shiner seaperch, (Cymatogaster aggregata), by Cushing and Beaver (1955), but the details of the method must apparently be adapted to each different species being studied. We have successfully preserved sockeye salmon red cells by a modification of this method for periods in excess of a month.

In conclusion, some of the advantages of using red cell antigens in racial studies are listed:

1. Whenever studied, the blood cell antigens have been shown to be genetically controlled. Variations in environment do not affect the blood type

2 The frequencies of occurrence of genes controlling cellular antigens have been found to differ significantly between races of man and between breeds of cattle. By means of these frequencies, races may be characterized or defined.

3 Once sufficiently potent antisera are prepared, the test for blood cell antigens is a relatively simple operation which could be carried out in the field or on board ship.

4. Commercially caught fish could be tested for their complement of blood cell antigens without damage to their commercial value since only a small amount of blood is required.

5. When unit serological reagents have been prepared, they would be of value as markers in studying the genetics of fish, the migration of fish and the precision of their homing abilities.

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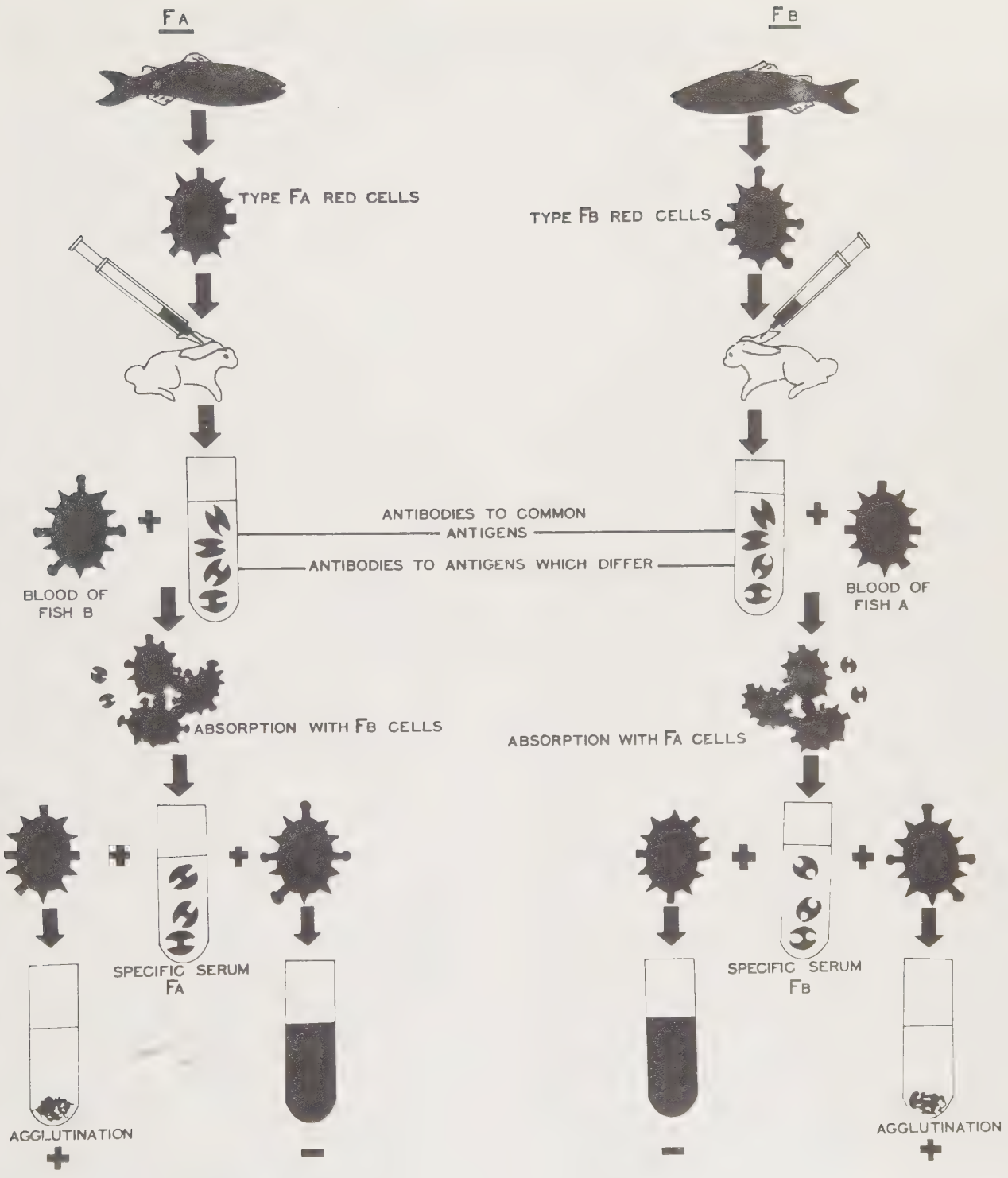

Figure 1.--Diagrammatic representation of the method used to discover antigenic differences in red blood cells.

A REVIEW OF RECENT STUDIES OF SUBPOPULATIONS OF PACIFIC FISHES

By

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INTRODUCTION

The chief objective of "population" studies has been to determine the extent of heterogeneity within the range of widely distributed species. Is a population composed of two or more groups that maintain a high degree of separateness, or is there mixing throughout the range of a species? If a population is made up of several groups that can be shown to possess structural differences, are the differences due to environment or to heredity?

According to the definition of a subpopulation given by Marr in the first article in this series, a subpopulation is a fraction of a population that is itself genetically self-sustaining. Hence, even to establish that a subpopulation exists, it is imperative to distinguish between the effects of environment and differences in heredity.

Actually, this has not been done for any species that will be considered in this paper. Hence, in a strict sense we shall not be dealing with studies of subpopulations, but rather with studies of population heterogeneity. In fact, as was frankly expressed by Rounsefell and Dahlgren (1935) in the paper on "races" of herring in southeastern Alaska "whether the differences in the characters chosen are due to heredity or to environment has not been considered as being of great importance, as long as the characters are fairly stable within each population so that significant differences indicate very slight intermingling, if any, between adjacent stocks of herring." (ibid:120). For convenience, I refer to the studies under review as "subpopulations" studies. Distinguishing "subpopulations" was clearly the intent if not the accomplishment of most workers.

There are two common approaches to the problem of recognizing subpopulations. One is an indirect approach and employs average morphometric or meristic differences between groups of fish to determine their probable separateness. The other is a direct approach and uses marked (tagged) members to assess the extent of mixing between groups of fish. The morphometric approach has been widely used because it can be done simply and inexpensively. Often the specimens to be studied can be obtained from the commercial catch. An effective tagging program requires a considerable outlay in money and personnel.

The hypothesis underlying morphometric and meristic studies of subpopulations of fish is simply this: under conditions of partial or complete isolation of groups of fish, slight differences in body proportions or meristic characters will be preserved in each group. These small differences will not necessarily be apparent in individual specimens but often only in an average of a large number of specimens. The significance of the differences is appraised by means of statistical procedures based on the theory of probability. The differences might be due to either environmental or hereditary factors. It is usually extremely difficult to determine whether differences are phenotypic or genotypic, yet knowledge of the cause(s) of the differences is essential to an understanding of their significance.

One of the critical points in this approach is one of interpretation. It has often been assumed by workers that if two groups of fish can be shown to differ significantly in one or several morphometric characters, the groups therefore are distinct with only slight intermixture. This conclusion has been reached in many of the papers

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reviewed in this article. But it has been found for several species for which both morphometric and tagging data are available that groups that were judged to be "distinct" on the basis of morphometric studies were found to have considerable intermixture when a tagging experiment was performed (Tester, 1948; Roedel, 1952). It is now realized that morphometric differences are evidence that a population is not homogeneous throughout its range; but the amount of intermixture can only be determined if certain special conditions are satisfied by the data. The problem is discussed by Royce and by Widrig and Taft in other papers in this series.

This paper will review some of the work done on "subpopulations" of Pacific marine fishes. The species which will be dealt with are the Pacific herring, Clupea pallasi; yellowfin tuna, Neothunnus macropterus; albacore, Germo alalunga; Pacific mackerel, Pneumatophorus diego; northern anchovy, Engraulis mordax; anchovetta, Cetengraulis mysticetus; nehu, Stolephorus purpureus and Australian anchovy, Engraulis australis. Subpopulation studies on the Pacific sardine are dealt with in another paper in this series; the Pacific halibut is omitted because the "studies of racial characters" mentioned in the introductory report on Pacific halibut (Thompson and Herrington, 1930: 11)[1/] have never been published.

In the papers which will be reviewed, there has been no consistency in terminology. Most commonly, the studies have been called "racial" studies. The term "population" has been used usually to designate the aggregate of individuals in particular localities; no worker has restricted the term to the definition given by Marr. There are similar differences in meaning attached to other terms, particularly to "stock and "race". In reviewing research done on "subpopulations" of Pacific fishes I have found it expedient to employ at times the terminology used by the authors of the papers being discussed.

1/ Tag returns from the early tagging experiments on Pacific halibut (tagging in 1925 and 1926 south of Cape Spencer, 1926 and 1927 north and west of Cape Spencer) showed that the immature fish tagged to the south of Cape Spencer were recovered near the areas of release with an average distance of recovery spot from release spot of only 22 miles. The mature fish tagged on the Yakutat spawning grounds and Portlock Bank were recovered 250 miles from the tagging spot, on the average. Only one fish tagged to the south of Cape Spencer was recaptured above Cape Spencer, and about 5 percent of the fish tagged in the western area were recaptured below Cape Spencer; evidence of the heterogeneity of the halibut stocks.

STUDIES DEALING WITH "SUBPOPULATIONS" OF PACIFIC COAST FISHES

Pacific herring, Clupea pallasi

The Pacific Herring is, in many respects, an ideal species on which to conduct subpopulation studies. The species is a demersal spawner, depositing its eggs on seaweed and eel grass near shore. The fish are thus concentrated at the time of spawning and can be caught and tagged on the spawning grounds. The species migrates offshore or along shore to feed, and in British Columbia at least, the herring is not fished during the feeding period. The fishery takes place during the inshore return of the herring to the spawning grounds. If the spawning runs maintain a high degree of integrity, the herring population could be made up of a large number of separate stocks.

The early work on "races" of herring attempted to show separateness of herring stocks by structural differences in meristic and morphometric characters. In this approach, American investigators were following techniques that had been pioneered by European workers in "racial" studies of Atlantic herring. The indirect approach was used because of the difficulty in conducting tagging experiments on herring. Marking experiments on herring were considered impractical, both because of the high mortality that would result from handling them in tagging, and because of the difficulty of recovering tagged members in a fishery where fish could not be individually examined on recapture (most herring are reduced to fish meal and oil).

The problem of an effective means of tagging herring and recovering the tags was finally

solved by Rounsefell and Dahlgren, who devised an internal "iron" tag that could be mechanically recovered in meal lines by electromagnets. Since its introduction, the internal tag has been widely used in subpopulation studies of Pacific Coast fishes, especially in studies on Pacific herring, Pacific sardine and Pacific mackerel.

The earliest paper dealing with "racial" variation in the Pacific herring is by Hubbs(1925). He summarized data on fin rays (dorsal and anal) and variation in number of vertebrae, based on counts made by Thompson (1917) and himself. All fish dealt with were either sampled in British Columbia or California Hubbs pointed out that there is a southward decrease in number of vertebrae in the Pacific herring.

A much larger mass of information is presented by Rounsefell (1930). He chose four characters to show structural differences: counts of vertebrae, of dorsal rays, of anal rays, and measurements of head length. Rounsefell examined samples from many localities between San Diego Bay in southern California and Golovin Bay in the northern part of the Bering Sea.

Variation in number of vertebrae.--The range in number of vertebrae in the Pacific herring is large, amounting to a difference of 13 between the lowest and highest counts recorded in the literature (46 to 58 including the urostyle). There is a marked difference in the average number of vertebrae in different localities. The lowest mean value, 51.68 vertebrae, is in fish from San Diego Bay, at the southern end of the distribution of the Pacific herring. The average number of vertebrae gradually increases to the north and westward, reaching highest average values along the Alaskan peninsula (Rounsefell, 1930). The highest average value recorded, 55.67 vertebrae, is in fish from the Shumagin Islands. The difference in mean vertebral number between fish from San Diego Bay and the Shumagin Islands amounts to nearly four vertebrae. Data on variation in number of vertebrae in different parts of the range of this species along the west coast of North America is summarized in table 1. Although this table is based primarily on data given in Rounsefell (1930) it includes counts of other workers. The source is indicated for each locality. For several localities the counts of two or more investigators are given.

Rounsefell summarized vertebral data for 32 localities in all: 5 localities between San Diego and British Columbia, 7 localities in southeast Alaska, 15 localities in central Alaska, and 5 localities in western Alaska. It is obvious that the extreme variations, such as that between San Diego and the Shumagin Islands, are significantly different. The heterogeneity of the Alaskan populations is also evident. The difference between the mean values for Stephen Passage in southeast Alaska and the Shumagin Islands off the Alaskan Peninsula of 2.31 vertebrae is 68 times the probable error of the difference.2/

When stocks of adjacent localities were compared, Rounsefell found what he considered to be significant differences in some instances. Thus, in southeast Alaska, Stephens Passage herring differed from samples from Chatham Strait localities by about 12 times the probable error of the difference between the vertebral means. On the other hand, the three localities in Chatham Strait did not differ significantly from one another.

2/ I have used the formulae given by Royce (formula 5 under concept of overlap) to compare three localities: San Diego Bay, Stephens Passage in southeast Alaska, and the Shumagin Islands. The overlap in distributions of the number of vertebrae in the several localities was as follows:

Locality	Percentage of overlap in vertebral counts
San Diego Bay - Shumagin Island	4
San Diego Bay - Stephens Passage	52
Stephens Passage - Shumagin Island	37

These values can be assumed to represent the maximum amount of intermingling that could take place between the herring groups in any two localities. The actual values of intermingling could be as low as 0, or some intermediate value between 0 and the above maximal values.

Table 1.--Variation in number of vertebrae in Pacific herring

Locality	No. fish	No. samples	Range in vertebrae	Mean or range of means	Source
San Diego Bay	408	1	50-54	51.68	Rounsefell 1930, table 8
Monterey Bay	89	1	50-54	52.03	Hubbs 1925, table 3
San Francisco Bay	735	2	48-54	51.77-51.83	Hubbs 1925, table 3
Puget Sound	100	1	49-54	52.71	Rounsefell 1930, table 8
Puget Sound	4,591	22	49-55	52.71	Chapman, Katz & Erickson, 1941
British Columbia	1,055	5	50-55	52.75-52.83	Thompson (1917)
B.C. (all areas)	13,598	91	49-56	52.68-53.40	Tester 1937, table 3
B.C. (west coast Vancouver Is)	22,087	60	49-57	52.66-53.07	Tester 1949 table III
S. E. Alaska	2,879	15	48-57	53.28-53.92	Rounsefell 1930, table 9
S. E. Alaska	10,753	54	48-56	52.96-53.80	Rounsefell & Dahlgren, 1935 table 3
Central Alaska (14 localities)	3,679	53	(48) 50-57	53.41-54.00	Rounsefell 1930, table 10 & 11
Shumagin Islands	456	1	52-58	55.67	Rounsefell 1930, table 12
Golovin Bay (64°30' N.Lat.)	140	1	52-55	53.79	Rounsefell 1930, table 12

Rounsefell found what he considered to be significant differences between samples from the same area. The variation was largely due to differences between year classes. The 1921 year class in McClure Bay (Prince William Sound area) differed from the 1923 year class by 0.67 vertebra. This was the extreme variation found. Samples composed of several year classes did not differ as much; the greatest variation found between such samples was 0.26 vertebra (Larch Bay, S.E. Alaska). When considering the significance of differences between two localities, it is essential to take into account the variation found between samples in the same locality.

On the basis of vertebral counts, Rounsefell postulated the distinctness of the populations in the following areas studied: California; southern British Columbia; three areas in S.E. Alaska (Craig, Chatham Strait and Stephens Passage); three areas in central Alaska (Cook Inlet-Shuyak Strait, Prince William Sound, Shearwater Bay - Old Harbor); and four areas in western Alaska (Chignik, Shumagin Islands, Unalaska and Golovin Bay).

Dorsal-ray counts.--The Pacific herring has from 15 to 21 dorsal rays with 17 to 20 being the usual number. Mean number of dorsal rays in fish from 9 localities between Puget Sound and Unalaska ranged from 18.70 to 19.05 (1 small sample had a mean of 19.36). The number of dorsal rays does not exhibit any general change with latitude, as do vertebrae, but appears to

vary independently of geographic location. Rounsefell did not find this character of use in showing the distinctness of any population.

Anal-ray counts.--The range in counts of herring anal rays found by Rounsefell was from 14 to 20; mean values in fish from different localities ranged from 16.61 (Puget Sound) to 17.23 (Dogfish Bay). Rounsefell considered that the evidence from anal-ray counts was inconclusive. As with dorsal-ray counts, there is no general change associated with latitude. There is as great a tendency for large differences between adjacent localities as between distant ones: For example, Dogfish Bay, with a mean count of 17.23, is adjacent to Shuyak Strait with a mean count of 16.71. The difference between two samples from one locality (Elrington Passage) was 0.35 ray, a variation as large as between most of the localities sampled by Rounsefell.

Difference in head length.--The only morphometric character used by Rounsefell (1930) was head length. He found that head lengths were smaller proportionately in large fish than in small, and he concluded that the length of head "does not increase as rapidly as the length of the fish," (ibid:267). He expressed head lengths as percentages of body length and compared curves of these percentages plotted against standard length; he was not able to express mathematically the differences between the regression curves. Subsequently, a covariance method of comparing morphometric measurements has been developed (Mottley 1941), that permits an assessment of the significance of the differences between regression curves. As a matter of interest, regressions were made of head length on standard length for two localities, "Southeastern Alaska total" and "Prince William Sound", using data given in Rounsefell (ibid: table 25). Only average values based on five or more observations were used.

Both regressions are rectilinear with little variation about the fitted lines (sy.x for both lines is only 0.3 mm). Statistics describing the two regressions follow: (below)

A linear relationship exists between the rate of growth of the head and the standard length. (See in this regard, Marr (1955:29). A difference in growth of herring as compared to trout may be noted; Mottley (1941) found it necessary to use logarithms of the measurements when dealing with regressions of head length on standard length in trout. A simple linear relationship between head length and standard length has also been found for yellowfin tuna (Schaefer 1948), Pacific mackerel (Roedel, 1952), and other fishes.

Herring "stocks" in southeastern Alaska.--Rounsefell and Dahlgren (1932) pointed out that a high negative correlation exists between the average temperature during the developmental period and the average number of vertebrae in different year classes. Hence, it is important when comparing samples of herring to deal with individual year classes rather than composite samples. Rounsefell and Dahlgren (1935) analyzed the "races" of herring in southeastern Alaska in greater detail than was possible in Rounsefell's earlier paper.

They studied material from 32 localities in southeastern Alaska. The chief character used was counts of vertebrae, determined by year classes for each locality. Other characters employed were rates of growth and relative abundance of year classes. Homogeneity of the material was shown for individual localities. A comparison was made of counts from each locality with those of adjacent localities, using fish of the same year class. The basic data of vertebral distribution by year class and locality are given in table 2. The authors list 71 comparisons of which 53 were considered not to show differences, 5 were approaching statistical significance and 13 were considered by the investigator to be statistically significant. Five of the statistically significant comparisons are between the 1926 class in Petersburg and the following localities: Vicinity of Juneau, Point Gardner, Meade Pt., Warren Island and Wrangell; three are between Noyes Island and localities just north of Cape Lynch (i.e., Coronation Island and Warren Island). Two of the statistically significant comparisons are between Cape

Locality	Size range (mm.)	Av. std. length	Av. head length	b	a	sy.x
S.E. Alaska	110-249	174.5	41.41	0.1970	7.0312	0.2998
Prince Wm. Sound	130-274	204.5	45.04	0.1774	8.7617	0.3013

Table 2.--Average number of vertebrae in herring from southeast Alaska, grouped by year-class and locality (based on Rounsefell and Dahlgren 1935)

Locality	Year-class						
	1923	1924	1925	1926	1927	1928	1929
Juneau - Icy Strait							
Pt. Adolphus				53.26	53.29		
Hoonah				53.42	53.40	53.80	
Pt. Augusta				53.34	53.54		
Vicinity of Juneau	53.37	53.39	53.33	53.38	53.44		
Todd - Peril Strait							
Todd					53.39		
Sitka - Chatham Strait & Vicinity							
Sitka Sound	53.78	53.60		53.38			
Point Gardner				53.47			
Meade Point				53.61			
Cape Bendel							53.30
Gut Bay, Chatham Strait					53.30	53.48	53.25
Deep Cove, Chatham Strait				53.27			
Port Herbert, Chatham Strait				53.33	53.28		
Tebenkof Bay, Chatham Strait			53.57	53.42			
Big Port Walter, Chatham Strait				53.48			
Cape Ommaney			53.69	53.42	53.63		
Coronation Island				53.46			
Kell Bay (Affleck Canal)				53.23			
Warren Island				53.36	53.59		
Favorite Bay, Kootznahoo Inlet				53.51			
Jamestown Bay, Sitka				53.41			
West Coast - Prince of Wales Island							
Noyes Island				53.19	53.34		
Culebra Island					53.35		
Klawak					53.25		
Port Estrella				53.11			
Inner Area - Southeast Alaska							
Wrangell		53.05	53.17	52.96	53.42		
Anita Bay					53.51	53.26	
Santa Ana Inlet						53.26	
Frances Cove						53.22	
Vicinity of Petersburg							
Vicinity of Petersburg			53.28	53.18	53.49		

Ommaney and localities near by in Chatham Strait (Port Herbert and Gut Bay, 1927 class).

Growth rates were analyzed by using length frequency distributions of individual year-classes by locality. Herring from four localities (Noyes Island area, the Juneau-Icy Strait area, Affleck Canal (Kell Bay) and Todd, Peril Strait) were found to be much slower growing than those of other localities. The Peril Strait herring appear to be the slowest growing of any encountered in Alaska.

Differences in relative proportions of different year-classes in localities, if sufficiently marked, may be used as evidence of non-intermingling of the stocks. Most localities were characterized by an overwhelming dominance of the 1926-class in both 1929 and 1930 samples. Marked differences in relative proportions of year classes were found in the following localities: (1) Noyes Island with an approximate equality of the 1926- and 1927-classes, (2) Peril Strait (Todd) with a marked dominance of the 1927-class (10 times as strong as the 1926-class), and (3) Douglas Island, Vicinity of Juneau, with a large preponderance of older fish and actual dominance of the 1923-class (twice as strong as the 1926-class in 1929).

Using three lines of evidence (vertebral counts, growth rates and relative proportions of year-classes) the authors found at least six areas to have independent populations. These are:

1. Juneau - Icy Strait area - on basis of growth rate and age composition.

2. Sitka - Cape Ommaney - Chatham Strait area, the center of the herring fishery in southeast Alaska.

3. Noyes Island and West Coast of Prince of Wales Island separated from even such near localities as Warren Island and Coronation Island on the basis of vertebral counts, growth rate and age composition.

4. Inner area of Southeastern Alaska - Wrangell, Anita Bay, etc., on basis of vertebral counts.

5. Vicinity of Petersburg - on basis of vertebral counts.

6. Todd, Peril Strait - on basis of growth rate and age composition.

The three main spawning grounds in southeast Alaska are located in the first three areas: The spawning area of the Juneau - Icy Strait herring is in the vicinity of Juneau. The spawning grounds of the central group are in Sitka Sound and the feeding grounds of this group are in the Cape Ommaney - Chatham Strait area. The Noyes Island herring probably are spawned at Klawak Inlet (Prince of Wales Island).

Rounsefell and Dahlgren (1933) initiated tagging and tag-recovery methods for the Pacific herring. They tried a variety of tags, and found that two kinds held promise, opercle tags and belly tags. In their initial tagging in Alaska in 1932 and 1933, they tagged 4,295 fish with belly tags and 4,733 with opercle tags. Recoveries consisted of 108 belly tags and 17 opercle tags, clearly demonstrating the superiority of the former. Dahlgren (1936) reported on more extensive tagging with internal tags during 1934 and 1935. Tagging results through 1935 are summarized in table 3. Recoveries were principally made in the Cape Ommaney region, the center of the herring fishery in southeastern Alaska. A movement of fish from the spawning grounds in the vicinity of Sitka to feeding grounds around Cape Ommaney is clearly demonstrated. Failure to recover fish tagged at Cape Bendel by the Cape Ommaney fishery is indicative of a lack of mixing between herring groups in lower Chatham Strait and in Frederick Sound. Similarly, failure to recover fish tagged at Auke Bay near Juneau by the Cape Ommaney fishery is indicative of a lack of mixing between those two areas. However, less than a thousand fish were marked with internal tags at Cape Bendel and at Auke Bay, hence the results cannot be considered conclusive.

"Populations" of herring in British Columbia.--Tester (1937) reported on "populations" of herring in the coastal waters of British Columbia.

His study was based on samples from 19 localities from British Columbia coastal waters, divided as follows: seven localities in the Strait

Table 3.--Herring tagging and tag recoveries from S.E. Alaska (through 1935)

Locality of tagging	Date	Type of tag		Recoveries	
		Belly	Opercle	Belly	Opercle
Cape Ommaney	July 27, 1932	-	1,034	-	10
Cape Ommaney	Aug. 3-4, 1932	-	633	-	0
Cape Bendel	Aug. 17, 1932	996	824	0	0
Jamestown Bay (Sitka)	Apr. 21-25, 1933	2,499	1,470	108	7
Near Juneau	May 3-5, 1933	800	772	0	0
Jamestown Bay (Sitka)	Apr. 2-9, 1934	13,167	-	680 1/	-
Klawak Inlet (Craig) Prince of Wales Isl.	Mar. 24-26, 1934	8,394	-	231 2/	-
Vicinity of Sitka 3/	Mar. 28-Apr. 5, 1935	27,911	-	2,040	-
Klawak Inlet (Craig) Prince of Wales Isl.	Mar. 19-23, 1935	13,008	-	288	-
Total		66,775	4,733	3,347	17

1/ Includes 482 recoveries during 1934, 198 during 1935

2/ Includes 149 recoveries during 1934, 82 during 1935

3/ Vicinity of Sitka includes Jamestown Bay, Kalinin Bay and Redoubt Bay

of Georgia (east coast of Vancouver Island and along adjacent mainland), six along the west coast of Vancouver Island and six from the outer coast above Vancouver Island to the Alaskan border, including one locality in the Queen Charlotte Islands. The material was collected over a 4-year period.

Morphometric characters studied were the following: standard length, head length, and distance from snout to insertion of dorsal fin. Meristic characters studied included vertebral counts, keeled scale counts and pectoral fin-ray counts. In addition, age determinations, based on scales, were made. As in other studies on herring populations, principal emphasis was placed on variation in number of vertebrae in different sampling areas.

Tester found a variation in mean vertebral count with latitude which conformed with the general gradation found along the west coast of North America by Rounsefell. During the period investigated, herring at Barkley Sound (49°N.lat.) averaged approximately 52.8 vertebrae; those at Jap Inlet (54°N.lat.) averaged approximately 53.2 vertebrae, or 0.4 vertebra higher.

The variation in mean vertebral number with year class is summarized in table 4. For most localities, data are available for 4 to 7 year-classes. The extent of the variation found between year-classes in a locality (4 or more year-classes considered) was 0.42 vertebra as the extreme, 0.10 vertebra as the lowest. Tester marshalls data to show that variation in mean vertebral count of successive year-classes in the same locality is related in some way to variation in water temperatures during the spawning and early developmental periods.

Variance of samples grouped by year-classes for each locality gave proof of the heterogeneity of the material and hence of the presence of more than one "population" in British Columbia waters.

When vertebral counts were divided into abdominal and caudal counts, some differences were found that were not apparent from total counts. Thus, a significant and constant difference was found between Saltspring Island and Barkley Sound herring which was not apparent from a consideration of the total count. Similar results were obtained on comparing the count of the Saltspring Island material with that of other localities on the west coast of Vancouver Island.

Mean keeled scale counts, a character widely used in investigation of the Atlantic herring, were made by Tester on many of the samples studied. He found that in all seasons the counts in northern latitudes averaged lower than those in southern localities. The range in mean keeled scale counts was from 11.75 to 12.13. The mean counts from Barkley Sound were about 0.25 scale higher than counts from northern British Columbia localities.

Pectoral fin ray counts were made on herring from all localities sampled in 1932-33. Individual counts varied from 14 to 20 fin rays, mostly 17 or 18. Mean counts ranged from 17.55 to 17.68. No uniform variation was found with latitude. Tester concluded that this character does not appear to vary significantly among herring from different localities in British Columbia.

Differences in rate of growth were found between herring in the vicinity of Vancouver and those in northern localities, with the higher rate found in southern British Columbia.

Tester used the same methods for treating data on head length as Rounsefell (1930). He also found a gradual decrease in the mean percentage head length with increase in body length. He found no consistent difference in this character between the sexes. Differences were found with age when comparing three to six year old fish collected during several seasons. Regressions computed from his data in table XV, of head length on standard length have the following statistics:

Age in years	Size range in mm.	Av. std. length	Av. head length	b	a	sy.x
III	165-200	182.5	42.64	0 1761	10.4994	0.1317
IV	180-215	197.5	45.73	0.1694	12.2674	0.0696
V	190-220	205.0	47.32	0.1669	13.0951	0.0858
VI	195-225	210 0	48.38	0.1562	15.5730	0.1754

Table 4--Mean vertebral counts of herring, by year-class, in British Columbia waters[1/]

Locality	1926	1927	1928	1929	1930	1931	1932	Greatest diff.
East coast Vancouver Island and adjacent mainland								
Saltspring Island	52.80	52.79	52.83	52.78	52.85	52.74	52.94	0.16
Departure Bay			52.85	52.74	52.80	52.91	52.86	
		(52.90)	(52.81)	(52.85)				0.17
Nanoose Bay		52.68	53.00	52.85	--	52.83	52.96	
				(52.76)	(52.75)	(52.58)		0.42
Granite Bay						52.87	53.18	0.31
Pender Harbour						52.74		
					(52.71)	(52.71)		0.03
Point Grey		(52.83)	(53.04)	(53.19)	(53.05)	(52.77)	(52.98)	0.42
Hammond Bay						52.81		
West coast - Vancouver Island								
Barkley Sound	52.65	52.76	52.86	52.90	52.84	52.76	52.87	0.25
Sydney Inlet		52.82	52.75	52.88	52.96	52.74		0.22
Nootka Sound		52.76	52.91	52.95	53.00	52.78		0.24
Esperanza Inlet			52.74	53.00	53.02			0.28
Kyuquot Sound		52.93	52.95	52.94	52.91	52.84		0.11
Quatsino Sound		53.02	52.82	53.04	52.85	52.93	52.86	0.22
Northern British Columbia								
Bella Bella		53.10	53.07	53.04	--	53.04	53.15	0.11
Skidegate Inlet			53.11		53.22		53.23	0.12
Jap Inlet		53.33	53.19	53.23	53.19	53.01	53.37	0.36
Butler Cove			53.40	53.28	53.07			0.33
Prince Rupert Harbour				53.14		53.14	53.17	0.03
Pearl Harbour	53.24	53.34	53.25	53.24				0.10
Greatest difference	.59	.66	.66	.54	.51	.66	.51	

1/ Based on data given by Tester 1937, Table 3. Values in brackets based on samples of gill-netted fish, all others caught by purse seine.

All mean values are based on 25 or more individuals and over half of values are based on 100 or more counts.

All four are linear regressions having a constant rate of increase of head length to body length. The percentage decrease in head length with increase in body length, noted by Tester, results from the fact that the y-intercepts (a above) differ considerably from zero. Both the slopes and y-intercept of the four regressions differ from each other, but it is not possible to assess the significances of these differences without having the original data instead of average values for determining the regressions. Whether the differences are age connected or whether they reflect the variability in this character between year classes is a moot point.

From a consideration of the differences found in meristic and morphometric characters and from additional evidence from age composition, Tester concluded that "intermingling of the runs of herring in British Columbia is limited in extent and that the total stock is divided into a number of essentially discrete units or local populations. Differences in one or several characters segregates the populations in the following localities or groups of localities: Point Grey; Granite Bay; Saltspring Island-Departure Bay-Nanoose Bay; Barkley Sound-Sydney inlet; Nootka Sound-Kyuquot Sound; Quatsino Sound; Bella Bella; Jap inlet -Butler Cove-Pearl Harbour. There are indications from age composition that the Sydney inlet run also forms a local population. Similar evidence indicates the presence of a local population at Skidegate inlet and at Pearl Harbour. The status of Prince Rupert Harbour, Bella Coola, and Pender Harbour herring has not been established, although it is certain that the latter are not part of the Point Grey run.

"The designation of these runs or local populations does not preclude the possibility of a slight degree of intermingling between adjacent or closely-situated groups. Because of this possibility and also because of incomplete knowledge of the seasonal movements which seem to take place during summer feeding grounds and winter habitats, no attempt is made to define precisely the areas which they occupy. The possibility also exists that further investigation will reveal that any of these populations may be further divisible into two or more separate units." (Tester, 1937:143-44).

By the time this report had appeared, British Columbia had entered upon an extensive tagging program, which was to alter some of the conclusions given above.

Tagging in British Columbia waters.--Tagging of British Columbia herring, begun in 1936, has been continued to date without interruption. The results are found in a series of yearly publications listed in the bibliography. Internal tags of iron plated with nickel or silver of the type developed by Rounsefell and Dahlgren were used. A portion of the tags are recovered at the time of unloading by means of an electronic tag detector placed in a chute or conveyor, but the great majority are recovered from electro-magnets installed in the meal line of herring reduction plants. The former method developed by Dahlgren (1936) is by far the more valuable method of recovery, for in addition to the tag the fish itself is recovered and can be measured for the second time, aged, sexed, etc., and exact information can be obtained on area and date of recapture and rate of growth.

During the period 1936-37 through 1953-54 (the last report available) over 737,000 herring have been tagged in British Columbia waters. The great majority of these (over 90 percent) were tagged on the spawning grounds after the commercial season had ended and so are not subject to recapture by the commercial fleet for at least six months following tagging. The average recovery from spring tagged herring has been about 25.7 fish per 1,000 tagged during the first season after tagging, 7.70 fish per 1,000 tagged during the second season after tagging, and then 1.90, 0.80, and 0.20 fish per thousand tagged during the three subsequent seasons.

These recoveries are somewhat lower than those obtained from a tagging experiment on the Pacific sardine. The two experiments are comparable, as an internal tag was used in tagging sardine, and recoveries were dependent upon electro-magnets placed in fish meal lines.

The returns are markedly lower than those obtained on hardier, longer lived fish such as the Pacific halibut. Recoveries of halibut tagged in 1951, for example, amounted to 199 per 1,000 tagged after three seasons, and the recoveries

from the area of heaviest fishing amounted to 378 per 1,000 fish tagged. The comparisons follow:

Recoveries per 1,000 fish tagged

Species and locality	Year after tagging					
	1	2	3	4	5	Total
Pacific sardine[1/]						
Tagged off Baja California	13.6	13.2	7.3	2.0	0.8	36.9
Tagged off S. California	30.6	12.8	4.3	1.9	1.0	50.6
Tagged off C. California	29.4	10.6	3.5	1.4	0.4	45.3
British Columbia herring (1936-37 to 1951-52)	25.7	7.7	1.9	0.8	0.2	36.3
Pacific halibut[2/] (tagged in 1951)	87.6	65.5	46.1	--	--	199.2

1/ From data given in Clark & Janssen, Calif. Div. Fish & Game, Fish Bull. No. 61.

2/ From data given in Reports of the International Pacific Halibut Commission, Nos. 20, 21, and 22.

Taggings were carried out on all main herring stocks in British Columbia waters in order to determine the degree of discreteness of herring populations. A summary of the percent emigrating from and immigrating into the principal areas ("populations") is given in the following tabulation:

Locality	Total catch 1936-51 (in thousands of tons)	No. fish tagged on spawning grounds 1936-51	Percent emigrating	Percent immigrants in catch
Queen Charlotte Island	--	--	--	
Northern Mainland	293	21,174	22.6	32.8
Central Mainland--North Central	187	18,440	23.7	29.3
" " South " outer	274	36,081	17.9	21.5
" " " " inner	16	11,929	--	--
Upper East Coast of Vancouver Island	80	34,231	--	--
Middle " " " " "	123	89,615	45.5	28.3
Lower " " " " "	569	108,365	16.4	41.1
Lower West " " " "	243	104,171	28.5	31.9
Upper " " " "	188	96,345	30.8	22.1
	1,973			

There is a considerable mixture of herring stocks along the British Columbia coasts. About a fourth of the fish of each "population" emigrate to other areas. The amount of emigration from one locality to another varies with the distance separating the stocks. Emigration of herring stocks of the Lower East Coast of British Columbia is lower than for other areas, but immigration into this area is higher.

Herring "populations" along the west coast of Vancouver Island.--Tester (1949) summarized extensive data gathered on herring "populations" along the west coast of Vancouver Island: over 22,000 vertebral counts made on 12 consecutive year classes from five contiguous areas. He analyzed the data for variation between samples, between age groups, between year-classes and between areas. He then proceeded to examine the reliability of results in the light of tagging experiments.

Variation between samples.--The individual samples of a year-class taken in one area during one year were tested for evidence of significant variation between means. Only one series of samples, out of 64, showed significant heterogeneity between the means of samples. Inasmuch as at least one divergent series could be expected among such a large number of series due to chance if sampling from a homogeneous population, this instance was not accepted as evidence of heterogeneity. Rather, it was assumed that each series of samples was drawn from a homogeneous population in respect to vertebral number.

Variation between age groups.--Fish of the same area and year-class but taken in different seasons were compared to determine whether they could have been drawn from one population in respect to vertebral number. Out of a total of 52 series involving two or more age groups of the same year-class, only two were found to exhibit significant differences between means. The two cases could represent freakish samples drawn by chance from a homogeneous population, although Tester offers alternative explanations: He concluded, however, that he would accept the view that different ages of a year-class in an area were drawn from one population with respect to vertebral number.

Variation between year classes.--I am reproducing table IV from Tester's paper. The table presents in concise form the basic information on mean vertebral count according to year-class and area. From an inspection of the table it can be seen that significant differences occur between the means of year classes in each area.

Area	Highest mean count for a year class	Lowest mean count for a year class	Difference
23	52.94 (1929)	52.72 (1938)	0.22
24	53.00(1932)	52.70 (1934)	0.30
25	53.00 (1930)	52.66 (1934)	0.34
26	52.94 (1928)	52.68 (1934)	0.26
27	53.07 (1929)	52.77 (1934)	0.30

Tester had shown in a previous paper (Tester, 1938) that variation in mean vertebral counts of successive year-classes (1927 to 1935) at Barkley Sound was inversely correlated with annual variation in mean water temperature in March at William Head at the southern end of Vancouver Island. Using augmented data Tester found a highly significant negative correlation between variation in March water temperatures at William Head and variation in the mean vertebral count of west coast of Vancouver Island year-classes over the period 1927 to 1938. Hence, the correlation previously found for Barkley Sound was found to be general along the whole of the west coast of the island.

Variation between areas.--The unweighted border mean vertebral counts for areas (refer to table 5) show a slight graded increase from the southern area to the northern area. An analysis of variance was made using two criteria of classification; year-classes and areas. The results show a highly significant heterogeneity between the means of year-classes (which was anticipated) and a highly significant heterogeneity between the means for areas. According to Tester, "This shows that, with due allowance for variation between year-classes, more than one population in respect to vertebral number is present among the five areas along the west coast of Vancouver Island." (Ibid:415.) In this analysis the mean square for "interaction" was also found to be highly significant. Two explanations are advanced as to why the mean counts for each year-class and area failed to vary in the same manner as the border means; one presuming local aberrations in temperature conditions during the time of early development and the other presuming partial mixture or interchange between the runs to the five areas. No choice is made of these alternatives, but Tester warns that "the general conclusion that each west coast of Vancouver Island area (with the possible exception of one) has an essentially discrete population should be accepted with reservation as to the possibility that mixture, more extensive than 'limited' may take place occasionally" (ibid:416).

Tester then tested the reliability of the foregoing with results obtained from tagging and tag recovery experiments. To quote Tester,

Table 5.--Mean vertebral count according to year class and area, west coast of Vancouver Island (from Tester 1949, Table 4)[1/]

Year-class	Area					Unweighted border-mean for year-classes
	23	24	25	26	27	
1927	51.734	51.823	51.783	51.933	52.007	51.856
1928	51.861	51.748	51.876	51.936	51.829	51.850
1929	51.940	51.880	51.954	51.942	52,070	51.957
1930	51.837	51.955	52.000	51.912	51.904	51.922
1931	51.757	51.744	51.767	51.886	51.927	51.816
1932	51.899	52.000 1/	51.895	51.904	51.919	51.923
1933	51.915	51.833 1/	51.923	51.908	52.022	51.920
1934	51.736	51.700 1/	51.661	51.676	51.770	51.709
1935	51.877	51.905	51.840	51.873	51.987	51.896
1936	51.883	51.989	51.908	51.930	51.797	51.901
1937	51.861	51.940	51.877	51.953	51.963	51.919
1938	51.720	51.824	51.793	51.837	51.855	51.806
Unweighted border mean for areas	51.835	51.862	51.856	51.891	51.921	

1/ Based on 10 or less individual counts

> Tagging has shown that the runs of herring to the various sounds and inlets along the west coast of Vancouver island form a series of intergrading populations, each of which exhibit a definite 'homing tendency' but each of which has a relatively small degree of independence. Rough calculations indicate that on the average about 55% of the fish return to the area of spawning, but the remainder, 45%, disperse or 'wander' to other areas. If the five areas are grouped into 3 (Area 23-24, Area 25-26, and Area 27), each of which is separated by a prominent headland which might act as a barrier to mixing, the calculations indicate that on the average about 70% of the fish return to the area of spawning and 30% wander to other areas. Mixture is greatest between adjacent areas and tends to become progressively less the more widely-separated are the areas.
>
> The conclusions drawn from a consideration of vertebral material are essentially in accord with the above findings, except that the amount of mixing between areas is considerably greater than might have been anticipated from the term 'limited'....so much so that for practical purposes the series of intergrading 'units' were considered to constitute one major population. (ibid.417, 419.)

Royce in a paper in this series uses some of the data of Tester (1949) in showing a condition of nearly complete overlap in vertebral counts from samples of herring off Vancouver Island. He compares the northernmost and southernmost areas and found a p of 0.465. Expressed as $\int($ = 200 p), the amount of one sample which might belong to another specified sample, the value is 93 percent. Hence, mixture between the two areas could be as high as 93 percent.

Yellowfin tuna, Neothunnus macropterus

It is difficult to investigate the subpopulations of tunas when even the number and distribution of the species is a matter of controversy. The exceedingly widespread distribution of these pelagic fishes, together with their large size, have contributed to the complexity of the problem. Because of their size, tunas are poorly represented in museum collections and, as a consequence, series of specimens have not been available for comparative studies until quite recently.

Kishinouye (1923) laid an excellent foundation for the morphological study of scombroid fishes. He not only used external characters such as meristic counts and proportionate measurements, but detailed anatomical studies of internal organs, circulatory system, and skeletal structures. His methods have been continued in detailed studies of various tunas by Frade (1931), Godsil and Byers (1944), Godsil and Holmberg (1950), and other workers. Although this approach has aided in clarifying the taxonomy of tunas, the studies to date have not been comprehensive enough to settle the species problem. It is still a matter of controversy whether there is a single yellowfin tuna species of widespread distribution or a number of related species of yellowfin tunas with more localized distributions. Similarly, the taxonomy of bluefin tunas and of albacores is unsettled.

The population studies have been more detailed than similar studies attempted on other groups of fish. There are several reasons why this was necessary. Meristic characters, with the possible exception of gill raker counts, are not particularly useful in defining subpopulations of tunas which (except skipjack) almost invariably have 39 vertebra. Fin counts are more variable, but little more useful. The proportionate length of fins changes with size, as do a number of body dimensions. Furthermore, different body parts grow at different rates. To define the relation of each character to size it is necessary to examine fish in the entire size range available at any one locality. This permits the various body proportions to be expressed as the regression of one dimension on another or on total (fork) length.

Marr and Schaefer (1949) define the body dimensions used in describing tunas including no fewer than 27 meristic and morphometric characters. Even this represented a selected list of characters, chosen (1) because of prior use,

(2) for facility in measuring or counting, or (3) as a conscious selection of characters likely to show possible differences.

Schaefer (1948), in his initial paper on morphometric characteristics of Pacific yellowfin tunas, reported on morphometric data for 46 yellowfin tuna from the waters off Costa Rica. He gives data on 21 of the 27 characters defined by Marr and Schaefer. For each character, Schaefer determined the linear least-squares regression on the total length (or head length). For most characters studied, the regression of the character on total length was a rectilinear regression of the type $Y = a + bx$. For three characters, it was necessary to transform the original data (using logarithms for one or both of the measurements) in order to yield a rectilinear relationship. The rate of increase in the length of second dorsal and anal fins was found to be greater than that of total length, while the rate of growth of the pectoral fin was less than that of total length.

Schaefer and Walford (1950) compared yellowfin tunas of Angola and the Pacific Coast of Central America. Morphometric data on Angola yellowfin included all 27 characters defined by Marr and Schaefer. The regression of body parts on standard length yielded results very similar to that found for Costa Rican tuna. All characters had a linear relationship with total length except length of second dorsal, anal and pectoral fins.

The most striking difference exhibited by tunas of the two areas was in the length of the pectoral fin relative to the length of the body. In both regions the length of the pectoral fin relative to total length decreased as the size of the fish increased, but the rate of decrease was more rapid for Costa Rican yellowfin. Similarly marked differences were found between the relative growth of the second dorsal and anal fins. In both proportions the Atlantic specimens had relatively larger fins than the Pacific specimens. Regression of the following characters on total length was slightly but quite significantly different between the two regions: head length, distance from snout to first dorsal, from snout to second dorsal and from snout to anal fin. No significant differences were found in the other characters studied.

The technique used in comparing the regression lines of tuna from two regions is that of covariance analysis. It provides a precise answer to the question of whether two samples differ more than would be expected from chance variation in samples from the same population. If such a difference exists it is good evidence of the heterogeneity of the population. It is not evidence that the stocks are completely separate (although they may be), nor does the technique afford a means of assessing the extent to which stocks may intermingle. However, such techniques are described in the papers in this series by Royce and by Widrig and Taft.

Schaefer (1952) extended his study to the yellowfin tunas of Hawaiian waters. He found nearly as marked differences between Hawaiian and Costa Rican yellowfin as he had found between Angolan and Costa Rican specimens. In fact, in the relative length of the dorsal and anal fins he found Hawaiian yellowfin to differ more markedly from Costa Rican specimens than the Angolan specimens had differed from the Costa Rican.

A count of gill rakers on 188 Hawaiian tuna had a mean value of 29.66 with a standard error of 0.087. Counts on yellowfin of the American West Coast averaged 30.46 with a standard error of 0.116 for 115 specimens. There is no correlation between size of fish and gill raker count, hence these characters avoid the difficulties of regression analysis. Consequently, this character seems to offer good possibilities for racial analysis of yellowfin tuna (ibid: 371).

The regression coefficients of various dimensions on total length for the three regions are given in the table on the following page.

In Hawaiian material, head length and the distance from the snout to the various fin insertions increase more slowly, relative to toal length, than in Costa Rican material, while the caudal portion of the body grows faster. As a result, large specimens of Hawaiian yellowfin are more elongate in the posterior portion of the body than specimens of comparable size from Costa Rica.

Schaefer concludes: "There is no doubt that the two populations (Hawaii vs. Costa Rica) are to be regarded as distinct. The possibility of some mixing between them is not excluded,

Regression coefficients of various dimensions on total length

Character	Hawaiian material	Costa Rican material	Angolan material
Head length	.2257	.2350	.2238
Snout to 1st dorsal	.2482	.2635	.2419
" " 2nd "	.4691	.4768	.4584
" " anal	.5194	.5351	. 5021
" " ventrals	.2526	.....	.2474
Length pectorals (on log TL)	491.93	445.0	537.4
Log length 2nd dorsal (on log TL)	2.213	1.694	1.895
" " anal (on log TL)	2.289	1.832	2.008

but if any exists it must be sufficiently small to permit the two populations to maintain their characteristic differences" (ibid.:372).

Godsil also has done considerably work on "populations" of yellowfin tuna (Godsil 1948, Godsil and Greenhood 1951). He has dealt with fewer characters than Schaefer (five in addition to body length), but has examined a much larger amount of material. He studied 13 samples containing a total of 1,911 fish from the area regularly fished by the American tuna fleet (from Gorda Bank, Mexico to the Galapagos Is.).

Godsil decided that the regressions of the characters studied (head length and distance from snout to fin insertions) on total (fork)length were nearly but not quite linear. He got the best fit with the function $Y = a + bx + c/x$.

Godsil tested the homogeneity of his 13 "American" samples and found that they could not be considered a homogeneous population. He then tried to find if the heterogeneity was associated with geographical origin. If such were the case, duplicate samples from the same region should be homogeneous, and it should be possible to obtain homogeneity by a proper and natural grouping according to origin.

Three of his samples were obtained from the same locality off Costa Rica. This is an interesting group, for they were caught from the same locality that Schaefer obtained his Costa Rican specimens. The regression coefficients for regression of the five dimensions on total length are given for Costa Rican samples in table 6. On testing for homogeneity Godsil found that the regressions of three of the five dimensions were significantly different between samples. Samples obtained from the same locality, consequently, were found to differ as much as samples from widely separated regions, hence it was concluded that the heterogeneity of the data is due to causes other than geographic segregation.

Godsil and Greenhood (1951) have also studied "populations" of yellowfin tuna in the Central Pacific. They had measurements on 79 yellowfin from Hawaiian waters, 94 from off Palmyra and Fanning Islands (4-6° N.lat., 159-162° W.long.,) and 13 from off the Fiji Islands. As in the earlier paper, five characters were used: head length, and the distance from snout to fin insertion (1st D, 2nd D, anal and ventrals).

They found that the Central Pacific yellow fin were significantly different from eastern Pacific yellowfin in all characters studied. The regression lines from central Pacific stocks were below the regression lines for eastern Pacific yellowfin for all characters. They concluded that the central Pacific stocks of yellowfin were distinct from and non-intermingling with those of the eastern Pacific. There can be little doubt that the two stocks are not homogeneous, but the authors have not demonstrated that intermingling does not take place.

Royce (1952) has summarized information on stocks of yellowfin tuna in the Pacific. In addition to the data already discussed, he included morphometric measurements from a number of other areas including the waters around the Line

Table 6.--Regression coefficients for regressions of various dimensions on total length for samples of yellowfin taken off Costa Rica

Item	Godsil #4 Nov-Dec 1936	Godsil #5 Jan-Feb 1937	Godsil #12 April 1940	Schaefer Jan-Apr 1947
No spec.	387	350	27	46
Head length	.2377	.2412	.2800	.2350
Sn-1st dorsal	.2565	.2579	.3086	.2635
Sn-2nd dorsal	.4746	.4814	.5039	.4768
Sn-anal	.5366	.5434	.5591	.5351
Sn-ventrals	.2718	.2721	.3006	--

Islands, Phoenix Island, Society Islands, Caroline Islands, Philippine Islands and Japan.

The difficulties in analysis of morphometric data are emphasized by Royce He also stresses the fact that when yellowfin samples from different areas are compared in all possible ways by means of covariance analysis, highly significant differences result from most comparisons. This even applies to some comparisons between samples from adjacent areas. He is skeptical of conclusions based upon such analyses, especially in view of the fact that racial studies on other species which have shown highly significant statistical differences between stocks have been contradicted by tagging studies. The existence of a statistically significant difference, consequently, is evidence merely that populations of fish are not homogeneous. They are not completely mixed although there may be considerable mixing.

Royce obtained some interesting results by comparing tuna of the same length in different areas. He used 100 cm. fork length. He found a tendency for the samples collected along the Equator to form clines or gradients. For certain characters--head length, distance from snout to insertion of the unpaired fins (first dorsal, second dorsal, anal), and body depth--the measurements were greatest in the Eastern Pacific and least in the Caroline Islands area. A cline in the other direction is indicated for the size of fins--fish in the western Pacific having the longest pectoral, second dorsal and anal fins. The regularity of the clines is shown by plotting individual characters, such as head length, against longitude.

An index of racial likeness was computed by Royce for simultaneous comparison of a number of characters in the larger samples. He obtained values ranging from 2.3 to 456.4. Although there is no exact scale for interpreting the results, small values show closer association, larger values a greater divergence. The largest value of 456.4 was obtained from the comparison of samples from Costa Rica and Eastern Caroline Islands, the smallest of 2.3 from a comparison of samples from Eastern Caroline Islands with central Caroline Islands Samples from adjoining localities along the Pacific Equator have small coefficients. An exception is the comparison between the Marshalls and eastern Carolines, with a coefficient of 74.2. Most of the samples obtained some distance from the Equator are not related to other samples as closely as are adjacent samples from the Equatorial area. The Philippine samples, for example, are greatly divergent from the adjoining Caroline Islands area and more closely related to samples from the eastern Pacific.

Royce concludes "our samples have been taken from stocks, which, with the exception of certain adjoining areas in the equatorial Pacific, are at least semi-independent" (ibid :161)

The latest paper n the series comparing morphometric measurements of yellowfin tuna of different areas is by Schaefer (1955). He compares yellowfin from southeast Polynesia (Marquesa, Society, and Tuamotu Islands) with those he had previously reported on from Costa Rica and Hawaii. The Polynesian specimens are similar to yellowfin from Hawaii in having very long second dorsal and anal fins in fact, these fins are even longer on Polynesian specimens than on those

from Hawaii. The pectoral fins of Polynesian yellowfin are also much longer than those on yellowfin tuna of comparable size from Costa Rica and significantly longer than those from Hawaiian waters.

With respect to morphometric measurements of head length and snout to fin insertions, Polynesian specimens appear to be intermediate between specimens from Hawaii and Costa Rica. In several characters (head length, snout to insertion of first dorsal, snout to insertion of second dorsal) the Polynesian specimens do not differ significantly from Costa Rican material but are different from Hawaiian specimens. In one morphometric measurement, however, distance from snout to anal insertion, the Polynesian yellowfin differ significantly from Costa Rican specimens but do not differ from Hawaiian fish. Gill raker counts from Polynesian tuna are identical with those reported by Schaefer for Costa Rican material (average total count of 30.60 on first arch), but nearly a gill raker higher than average counts of 29.66 gill rakers found for Hawaiian yellowfin. This is further evidence of the heterogeneity of the yellowfin tuna stocks of the Pacific.

The extent of the intermingling of tuna stocks in the Pacific can only be determined from an extensive tagging program. Tuna tagging was tried by Godsil (1938) as early as 1934-38, when 4,000 yellowfin and skipjack were marked with a preopercular strap tag. Only a single yellowfin tuna was recaptured, one day after release. A tuna marking program was reactivated by members of the California Department of Fish and Game in early 1952, and the marking methods and recoveries were reported by Wilson (1953). During 1952 and 1953, 1,950 yellowfin and 590 skipjack were tagged with the newly developed streamer-type vinylite-tubing tags. There were 14 recoveries of yellowfin reported by Wilson, two of which were caught 204 and 253 days subsequent to tagging. The areas of release and recovery were not given. Although tuna tagging has been intensified since Wilson's report, there have been no further reports on the experiments.

Albacore, Germo alalunga

Godsil (1948) made morphometric comparisons of albacore from three areas of the north Pacific: from off the west coast of America, from the vicinity of the Hawaiian Islands and from the vicinity of Japan. Over 100 specimens of albacore from American waters were studied, but the sample from Hawaii consisted of only three fish and the samples from Japan of only nine fish. Fourteen morphometric measurements were made on each fish. The number of American specimens was sufficient for making regressions of each character on standard length, the few Japanese and Hawaiian specimens were simply compared with these regressions. Godsil found that the Japanese specimens differed significantly (probability below the 1% level) from the American specimens in ten of the 14 characters studied. The Japanese specimens had a shorter head and caudal region than the American material, the body was relatively deeper, the first dorsal fin was higher, the eye was larger, and the pectoral fin was proportionately longer. Godsil found the three Hawaiian specimens to be more closely related to the Japanese than to the local population. A conclusion arrived at by Godsil from his comparisons was the following: "The local and Japanese populations of albacore are probably distinct and non-intermingling."

There have been several attempts to trace the movements of albacore by tagging experiments. Godsil, during the tuna tagging program of 1934-38, tagged 70 albacore according to Wilson (1953). This was not mentioned by Godsil (1938). The fish were tagged on the preoperculum with a strap-type metal and celluloid tag. The Fisheries Research Board of Canada (British Columbia) tagged 140 albacore in 1948 and 355 in 1950. The first group was tagged with a celluloid button-type tag inserted in the caudal fin, the second group was marked with a hook tag. There have been no recoveries. The North Pacific Exploratory Program of the U. S. Fish and Wildlife Service tagged 621 albacore during 1950-1952 using Peterson-type tags and streamer tags. There have been no recoveries. Alverson and Chenoweth (1951) conducted water tunnel tests on different types of fish tags inserted on albacore. They found that streamer-type tags were the most successful of the kinds tested. The California Department of Fish and Game began marking albacore with vinylite-tubing tags in 1952. There have been a number of recoveries.

An albacore tagged on August 4, 1952 at

lat. 38°25'N., long. 118°15' W. (18 miles south of Los Angeles Harbor, California) was recovered south of Japan, at lat. 31°30', long. 149°40'E. on June 23, 1953 (Ganssle and Clemens 1953). The recovery locality was approximately 550 miles southeast of Tokyo. The tag was of vinylite tubing secured with nylon line. The specimen was one of 215 albacore tagged in August 1952. Two other specimens of the same group had been recaptured off Morro Bay, California, within 43 days from the time of tagging.

Two albacore tagged off northern Baja California in August 1953 were recaptured in the vicinity of Midway Island in the mid-Pacific (Blunt 1954). The first recovery was obtained at 36°40' N, 178° 12' E on February 2, 1954. The second albacore was caught on February 23, 1954 at 30°10' N Lat., 178°54' W. The tags on both specimens were made of vinylite tubing. The two specimens were from a group of 754 albacore tagged in the vicinity of Guadalupe Island. Twelve other specimens from the same group were recovered off central California within 45 days of release.

A news release from the Pacific Oceanic Fishery Investigations, dated February 6, 1956, reports that an albacore tagged October 5, 1954 at a point 1,300 miles north of the Hawaiian Islands was recaptured on January 19, 1956 about 75 miles northeast of the entrance to Tokyo Bay.

The above returns offer quite definite proof that albacore perform extensive migrations in the north Pacific. Whether this applies to the population as a whole, or only to a portion of the population, is not known. Here again tagging did not bear out the conclusion reached from morphometric comparisons, that local and Japanese albacore are non-intermingling.

Pacific mackerel, Pneumatophorus diego

One of the interesting population studies of Pacific fishes is that conducted on the Pacific mackerel, Pneumatophorus diego, reported by Roedel (1952). Meristic and morphometric comparisons were made on Pacific mackerel from a number of areas, and the results were evaluated in the light of tagging experiments.

Material was examined from the following geographic areas:

Locality	No. samples	No. specimens
British Columbia	2	141
Southern California	20	897
Soledad (Ensenada, northern Baja California)	2	196
Viscaino Region, central Baja California	6	460
Cape San Lucas Region	2	641
Gulf of California Region	4	242

Meristic counts.--Meristic counts were centered on variations in positions of structures of the vertebral column with respect to the number of the vertebra on which they first occurred. Variation in total number of vertebrae was of no value: total number of vertebrae is an exceedingly constant character in the Pacific mackerel. Out of 2,352 fish studied, only 10 specimens had other than 31 vertebrae: 3 specimens had 30 vertebrae and 7 had 32 vertebrae. But little more variation was found in the position of the first haemal spine; all but 40 individuals had the spine on the fifteenth vertebra, the remainder were either one more or less than this. The position of the first haemal arch was somewhat more variable, about 10 percent of the specimens had the arch on either the tenth or twelfth vertebrae, with the remainder of specimens having the first haemal arch on the eleventh vertebra. Differences among the four "northern" regions in this character were not significant, nor between the two southern regions (Cape and Gulf). Differences between Viscaino and the Cape San Lucas region were highly significant.

Roedel found a structure on the vertebrae, which he termed the haemal brace, to be the most variable meristic character. The haemal brace extends from the centrum to the haemal arch; it may be either paired (usually) or single on the vertebra of first occurrence. Variation found in the first occurrence of the haemal brace was as follows:

Frequency of first occurrence of the haemal brace on vertebrae Nos. 14 to 18

Locality	Vertebra number				
	14	15	16	17	18
British Columbia	12	76	53		
Southern California	73	444	360	5	1
Soledad	11	98	86	1	
Viscaino	33	229	196	1	
Cape	23	199	412	6	1
Gulf	9	117	115	1	
Totals	161	1,163	1,222	14	2

The differences between the four northern regions are not significant. Differences between Viscaino and Cape regions were "extreme" and differences between the two southern regions, Cape and Gulf, were pronounced. Differences between samples from the same area were not significant for four localities, were on the borderline of significance for southern California ($P = .01$) and were significant for Viscaino ($P .0001$). The variation in Viscaino was associated with differences between age classes, the divergent sample being of small fish of the year.

Morphometric comparisons.--Head and fork lengths were measured for most samples (not taken on British Columbia specimens). When head length was plotted against fork length, no departure from rectilinearity was found, and the regression lines were of the type $Y = a + bx$. Statistics describing the regressions of head length on fork length for each area are given in the following table.

Region	No samples	No. fish	Size range	Mean fork length	Mean head length	a	b	$S_{y.x}$
So. Calif.	5	206	183-373	256.14	68.99	5.62	.2474	1 17
Soledad	2	176	145-322	197.39	54.37	4.09	.2547	0 92
Viscaino	6	429	153-391	277.99	75.80	5.13	.2542	1 68
Cape	2	524	210-332	266.42	74.10	9.12	.2439	1.20
Gulf	2	148	248-366	326.13	90 13	1.98	.2703	1.79

Differences among southern California samples are significant. This could be interpreted as evidence for two or more genetically separable populations, or it could be considered to reflect differences between age groups and/or recruits from different spawning grounds. The samples from other regions are not different "within regions". However, all five regions were found to differ significantly from one another in head length; the differences are far greater than those associated with meristic characters. From this morphometric study Roedel concluded that each region was characterized by distinct groups of fish; those in California and Soledad with relatively short heads, those to the south with progressively longer heads.

In summary, fish from the four northern areas were not considered separable on the basis of vertebral characters. Head length data were not available for British Columbia. For the remaining three regions, the comparisons indicate that the fish were in all probability drawn from three statistically distinguishable stocks. Viscaino and Cape fish were sharply set apart in all respects. The Cape fish were characterized by a more posterior appearance of the vertebral structures and by relatively longer head. Gulf fish were strongly differentiated from Cape fish, and form a unit apart on the basis of Roedel's study.

Roedel did not find that these conclusions were compatible with evidence from tagging experiments (Fry and Roedel 1949). Tagged fish from Soledad and Viscaino regions were later recovered in both southern and central California. (No facilities were available for recovery in Baja California.) Returns from southern California-tagged fish were far more numerous than returns from Viscaino fish for the first two years after release, but in the third and fourth year after tagging returns from both regions were of similar magnitude. The separation of California and Viscaino stocks was not as complete as seemed indicated by the morphometric study. No British Columbia fish were tagged, but of 11 tagged near the mouth of the Columbia River, one was recaptured off southern California. No fish were tagged south of the Viscaino region.

Royce (this collection of papers) uses Roedel's data on morphometric comparisons of head length in illustrating the overlap of measured characters when regression has been used. He then compares the results with the tag return data, and does not find that the two kinds of data are incompatible. The reader is referred to Royce's paper.

Northern anchovy, Engraulis mordax

Until recently, the northern anchovy has been but little investigated. The systematic sampling of the commercial catch and age analyses were begun only in 1952. (Miller 1955, Miller et al 1955.) Anchovies have not been tagged, so nothing is known of the extent of their migrations or mixing over their distributional range, which extends from the Queen Charlotte Islands, British Columbia, to Cape San Lucas, Baja California.

Two studies have been made of meristic characters. Hubbs (1925) reported on variation in vertebral counts off central and southern California. He separated the population inhabiting the brackish waters of San Francisco Bay as a distinct subspecies, Engraulis mordax nanus Girard. The San Francisco Bay anchovy differs from the ocean form in having a lower vertebral count (av. of 43.80 as compared to 45.73 for the ocean form in the San Francisco region), smaller size, longer head and deeper body. The lower vertebral count found in the bay form parallels the situation found in the nehu in Ala Wai Canal, Oahu (Tester and Hiatt 1952), and in the Australian anchovy, Engraulis australis, in Gippsland Lakes rivers (Blackburn 1950).

McHugh (1951) reported on a study of Engraulis mordax mordax "designed to determine the extent of variation in space and time in morphological characters of the northern anchovy, and on the basis of these findings to interpret the population structure of the subspecies." (ibid.:124.) He studied the distribution in mean values of five meristic characters: vertebrae, anal fin rays, dorsal fin rays, pectoral fin rays and gill rakers.

The vertebral data grouped by stage of development and area are presented in table 7.

There is little difference in the mean number of vertebrae in the samples from British Columbia, Washington, Oregon and California (range 45.69 to 45.82 vertebrae), but there is a falling off in mean number in samples from Baja California, amounting to approximately 0.4 vertebrae off southern Baja California. (Turtle Bay to San Juanico Bay). McHugh concluded "there is thus no evidence from vertebral counts that racial differences occur along the entire Pacific coast of Canada and the United States. It appears, however, that intermingling between these areas and Baja California is either incomplete or lacking." (ibid.:131)

McHugh found differences between samples from the Pacific Northwest and southern California in mean fin ray counts. The differences were most marked in the mean number of anal fin rays. Individuals from southern California averaged over 0.5 ray higher than individuals from the Pacific Northwest. However, there was considerable variation in the means of samples from each area, as is apparent from the following tabulation:

Locality	Anal fin rays Range in mean values in samples
British Columbia	21.89 – 22.38
Washington	21.89 – 22.25
Oregon	21.89 – 22.43
Central California	22.22 – 22.60
Southern California	22.57 – 23.07
Baja California	22.08 – 23.00

Table 7.--Northern anchovy: mean number of vertebrae of adults, young and post larvae grouped by area

Locality	Adults		Young		Postlarvae		All ages	
	Mean	No.	Mean	No.	Mean	No.	Mean	No.
British Columbia	45.76	379	45.71	106	45.68	19	45.74	504
Washington	45.72	165	-	-	45.86	360	45.82	525
Oregon	45.71	301	45.81	147	45.72	29	45.75	477
No. California	45.80	167	45.71	83	45.65	37	45.75	287
So. California	45.70	531	45.79	152	45.67	1088	45.69	1771
No. Baja Calif. (to Point San Eugenio)	45.32	22	45.57	66	45.41	128	45.45	216
So. Baja Calif. (So. of Point San Eugenio)	45.59	93	45.10	10	45.25	460	45.31	563
								4343

McHugh found less difference in dorsal ray counts between areas. Again, the highest mean counts were obtained from southern California. The variation within an area, however, was considerably less than for mean values of anal fin rays. McHugh concluded "on the basis of dorsal fin ray counts, the northern anchovy may therefore be divided into at least two populations....Distinct populations inhabit the waters of the Pacific Northwest and of southern California and that these may be separated rather sharply off the central California coast, possibly somewhere in the vicinity of Point Conception. There are also indications that a third population exists off Baja California" (ibid.:142-3).

The mean number of pectoral fin rays was found to increase from north to south. McHugh found clear-cut sexual dimorphism in the number of fin rays, with males averaging higher than females in all fins studied. The dimorphism was most marked in mean number of pectoral rays, however. The counts on males exceeded those on females by 0.29 ray on the average.

Female anchovies were found to consistently exceed males in the mean number of gill rakers; for both sexes it was found that gill rakers increased gradually in number with increase in size. In studying latitudinal variation it was therefore necessary to compare individuals of the same size and sex. It was found that the mean number of gill rakers decreased from north to south.

Based on an analysis of all five meristic characters, McHugh concluded that "at least three populations inhabit the coast, occurring (1) from off British Columbia to northern California, (2) off southern California and northern Baja California, and (3) off central and southern Baja California."

It is necessary to check the above conclusions with tagging experiments. Differences between areas in all characters studied were so small that based on these evidences alone it would not be possible to show separateness of "populations" especially if the concept of overlap discussed by Royce were applied.

Anchovetta, Cetengraulis mysticetus

The anchovetta, Cetengraulis mysticetus, is the principal bait fish used by American tuna fishermen to catch yellowfin and skipjack tunas. Decrease in abundance of this species, especially in the Gulf of Nicoya, was one of the factors that led to the establishment of the Inter-American Tropical Tuna Commission. Howard (1954) reported on a study of "populations" of this anchovy, using meristic characters to determine whether more than one major population occurs in the range of the species from Mexico to Peru.

Specimens were examined from six major baiting localities covering nearly the entire range of the species. The localities were the following: Almejas Bay on the outer coast of Baja California

(at the northern end of the range of the species), Guaymas and Ahome Point in the Gulf of California, Gulf of Fonseca, Gulf of Panama, and Gulf of Guayaquil. Four meristic characters were studied: vertebrae, dorsal fin rays, anal fin rays and gill rakers. Vertebral counts were made on 250 fish from each locality and other characters were studied on 125 specimens from each locality. Unlike the northern anchovy, none of the meristic characters were found to vary with sex. Only gill-raker counts increased in number with increase in length of fish.

A summary of the mean number of vertebrae and fin rays for each locality follows:

Locality	Vertebrae	Dorsal fin rays	Anal fin rays
Almejas Bay	41.18	15.02	22.18
Guaymas	41.04	15.03	21.37
Ahome Point	41.04	15.13	21.61
Gulf of Fonseca	41.10	14.99	21.62
Gulf of Panama	41.06	15.07	21.98
Gulf of Guayaquil	41.05	15.09	22.06
All localities	41.08	15.05	21.80

The range in number of vertebrae was found to be small, 39 to 43, and in all localities the majority of anchovettas had 41 vertebrae. Only one locality, Almejas Bay, was found to differ significantly from the remaining localities with respect to vertebral number.

The dorsal ray counts ranged from 13 to 17, with over 70 percent of the anchovettas having 15 fin rays. No difference was found between localities. Anal fin ray counts ranged from 18 to 25 with most fish having 21 to 23 rays, but with variation associated with locality. From a comparison of the anal ray counts of each locality with those from the other localities it was concluded that two population groups could be identified: (1) Guaymas-Ahome-Fonseca and (2) Almejas-Panama-Guayaquil. The first group represent adjacent localities; Almejas, on the other hand, is widely separated from Panama and Guayaquil and was shown to be distinct from these in mean number of vertebrae.

From a study of the number of gill rakers on the first arch related to length, it was found that fish from Almejas Bay, Guaymas and Panama fall close to the average curve (all localities combined) whereas Guayaquil and Fonseca anchovettas have a smaller number of gill rakers, and Ahome Point fish have a larger number.

Considering all four meristic characters, Howard concludes that three population groups are indicated: (1) Almejas Bay, (2) Guaymas, Ahome Point and Fonseca, and (3) Panama and Guayaquil. He further postulates that the localities within groups (2) and (3) appear to be distinct from each other on the basis of gill raker counts, hence the six localities should be considered provisionally to have separate stocks of anchovettas. Although the analyses indicate that there is not free interchange of anchovettas between the six principal baiting areas, the possibility of partial intermixing is not precluded. To assess the extent of mixing, Howard suggests that a tagging experiment on fish in all important baiting areas should be undertaken. In the last annual report of the Inter-American Tropical Tuna Commission (annual report for the year 1954), a summary is given of a tagging experiment in the Gulf of Panama. About 5,000 anchovettas were tagged during 1954, of which 5 have been recovered subsequently, all within the Gulf of Panama. One recapture was made about 110 miles distant from the point of tagging. Tagging has not been started as yet in other areas.

Nehu, Stolephorus purpureus

A study of populations of the nehu, Stolephorus purpureus Fowler, using variation in vertebral number, was made by Tester and Hiatt (1952). The study was based on 18 samples collected in seven localities from three Hawaiian Islands, Oahu, Maui and Hawaii.

The nehu is characteristically an in shore fish, occurring for the most part in isolated localities where the water is less saline and more turbid than in the open sea. Thus, it is possible that a discrete population of nehu occurs in each baiting area.

The mean number of vertebrae and the range in means of the several samples from each locality are summarized in the following table:

Locality	No. fish	No. samples	Mean no. of vertebrae	Range in mean no. of vertebrae
Ala Wai Canal, Oahu	503	4	42.54	42.49-42.68
Honolulu Harbor, Oahu	303	3	43.55	43.35-43.81
Pearl Harbor, Oahu	196	2	43.37	43.20-43.50
Kaneohe Bay, Oahu	1115	3	43.64	43.57-43.75
Kihei, Maui	444	3	43.70	43.67-43.80
Hilo Bay, Hawaii	288	1	43.61	43.61
Hawaihe Bay, Hawaii	268	2	43.74	43.66-43.85

The data show a striking difference between the mean vertebral number of Ala Wai Canal nehu and those of other localities. The authors point out that the water in Ala Wai Canal is less saline than in other localities and they postulate that the outstandingly low mean count for Ala Wai Canal fish "is probably related in large part to the brackish-water habitat." (ibid.:68).

Ala Wai Canal is the only locality in which the existence of a separate stock has been demonstrated. The authors point out that the difference in mean vertebral counts between the other localities could have arisen in random sampling from one statistically complex biological population. However, they do not entirely dismiss the possibility that essentially discrete units occur in each baiting area, which might be demonstrated by more extensive sampling and other methods of study.

Australian anchovy, Engraulis australis

A study of the populations of Australian anchovy was made by Blackburn (1950). Using differences in average vertebral counts, he divided the Australian anchovy into three groups which he considered distinct enough to be classed as subspecies. These were:

1. Engraulis australis australis from Queensland and New South Wales, having a range of 40 to 46 vertebrae and average counts in different localities of 43.10 to 43.99 vertebrae. Average dorsal fin ray counts ranged from 14.07 to 14.60.

2. Engraulis australis antipodum from Twofold Bay through Victorian, Tasmanian and probably most South Australian waters, having a range of 42 to 48 vertebrae and means from 44.90 to 45.84. Average dorsal fin ray counts ranged from 14.47 to 15.31.

3. Engraulis australis fraseri from Western Australia, having a range of 41 to 46 vertebrae and means from the two localities studied of 43.12 and 43.83 vertebrae. The average dorsal fin ray count in one of these localities was 14.49.

Blackburn points out that the number of vertebrae and dorsal fin rays increases toward higher (colder) latitudes. The group he separates as Engraulis australis antipodum occurs to the south of the other two subspecies. Hence, it is possible that the differences found by Blackburn could be due to environmental factors alone.

The differences are certainly markedly less than those found in the Pacific herring (51.68 vertebrae in San Diego Bay as compared to 55.67 vertebrae in samples from the Shumagin Islands as reported by Rounsefell 1930), yet no similar subdivision of the herring populations into subspecies has been made.

In a similar study of the Australian pilchard, Blackburn (1951) differentiated three major groups which he designated as "races". The eastern and southeastern groups of pilchards were delineated like the corresponding groups of anchovies; the third group of pilchards occupied waters off southern Western Australia. The three "races" were separated on the basis of growth rate and spawning seasons. The eastern and southeastern races were subdivided into smaller, more or less separate stocks. A major difficulty in studying subpopulations in Australian pilchard and anchovy has been the virtual absence of a commercial fishery for these species.

SUMMARY

In this paper I have reviewed research on "subpopulations" of eight Pacific fishes. The species dealt with were the following:

Pacific herring	(Clupea pallasi)
Yellowfin tuna	(Neothunnus macropterus)
Albacore	(Germo alalunga)
Pacific mackerel	(Pneumatophorus diego)
Northern anchovy	(Engraulis mordax)
Anchovetta	(Cetengraulis mysticetus)
Nehu	(Stolephorus purpureus)
Australian anchovy	(Engraulis australis)

I have summarized the techniques used in studying subpopulations of the species discussed in this paper in the following table:

Species	Meristic characters						Morphometric characters				Tagging
	Vertebra	Gill rakers	Fins D	Fins A	Fins P	Other	Head length	Snout to fin insertions	Fin length	Other	
Pacific Herring	x		x	x	x	x	x	x			x
Yellowfin tuna		x					x	x	x	x	x
Albacore							x	x	x	x	x
Pacific mackerel	x					x	x				x
Northern anchovy	x	x	x	x	x						
Anchovetta	x	x	x	x							x
Nehu	x										
Australian anchovy	x		x								

In none of the studies were the workers able to distinguish between differences induced by the environment and those which had a genetical basis. Hence, the workers were dealing with "groups" as defined by Marr, rather than with "subpopulations" and "stocks". For all species, the indirect approach has been utilized (meristic and/or morphometric characters), and for five of the species some tagging has been done. However, for only two species has tagging been extensive enough to be of value in subpopulation studies, i.e., Pacific herring and Pacific mackerel.

In studying subpopulations of clupeid and engraulid fishes, most workers have relied on meristic characters, especially vertebral counts. In studying subpopulations of scombroid fishes (tunas and mackerels), on the other hand, meristic characters have shown too little variation to be of value, hence techniques have been developed for utilizing morphometric characters.

For all species studied it has been possible to show "population heterogeneity". However, it has not been possible to determine the extent of separateness of the various parts of a population by the indirect approach. Even such advances in this field as the "concept of overlap" discussed in the paper by Royce do not entirely resolve this difficulty. For determining the extent of intermingling there appears to be no substitute for effective tagging experiments.

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RACES OF CUTTHROAT TROUT IN YELLOWSTONE LAKE

By

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Most trout populations in the United States are not well suited for studies on races because of our lack of knowledge of their identities. Since before the turn of the century, American fishery workers have energetically introduced trouts into barren waters and added to already existing populations. This has resulted in hybridization between species, interbreeding of subspecies, and the mixing of races, both in nature and in fish cultural establishments. Records have been poorly kept or lost in many instances, so the resulting trout populations in most of our waters are not accurately known as to origin, and studies on established races would be most difficult to approach.

In many cases where exact identities are known, the trout have been introduced so recently that there has not been sufficient time for races to separate or form. In other instances where pure stocks were recognized, they have recently become extinct, as with the Utah cutthroat, Salmo clarki utah Suckley, and the Lahontan cutthroat, Salmo clarki henshawi Gill & Jordan (Miller, 1950).

Yellowstone Lake in Wyoming supports a stock of the Yellowstone cutthroat, Salmo clarki lewisi (Girard), that has remained free of the influences of any trout from outside the drainage. No other species of trout have been introduced, and no cutthroat from other waters have entered the lake or its tributaries, save perhaps a small number of Snake River cutthroat that may have wandered in through the original avenue of natural stocking.

The cutthroat in Yellowstone Lake, then, is admirably fitted for studies on its races. The stock has been virtually pure for a long time, the geography is well adapted for such studies, and enough biological investigation has been carried on to provide a moderate accumulation of facts on which to base an inquiry into the formation and existence of races.

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TERMINOLOGY

Many terms have been used to designate taxonomic groups of animals below the species level. The subspecies is the most widely used such designation in zoology, and is defined by Mayr, Linsley, and Usinger (1953) as "geographically isolated aggregates of local populations which differ taxonomically from other such subdivisions of a species." Other names are sometimes used synonymously with the term "subspecies", the term "race" being in use by many groups of zoologists. Fish terminology often utilizes the "race" in designating populations within the subspecies, and it is in this sense that the name "race , or "local race" is used here. Other names, such as "sub-population, might be used, but not the term "subspecies", since it is obvious that we are dealing with separate populations within a group considered by ichthyologists to be a valid subspecies.

Races have been described in several ways. The "geographical race" is localized geographically, and the "ecological race" is localized ecologically. However, since no two localities are alike ecologically (Mayr, et al, 1953), the two are alike. "Physiological races" are those which differ from related populations in some physiological way, as with "temperature races" Temperature races are those having particular temperature preferences or tolerances, and are common in fish, whose dependence upon the temperature of the environment is greater than that of warm-blooded animals. Other physiological races differ from their close relatives

in factors of sex and reproduction, and are known as "sex races". Ecological races of other kinds have been described as "seasonal races" which cannot mate with other races because the times of reproduction do not coincide, and "altitudinal races", which are races separated altitudinally from others.

Salmo clarki lewisi in Yellowstone Lake probably is divided into races of several of the kinds noted above. It may be that races should not be differentiated so delicately, since many are considered to be synonymous. The fact remains, however, that factors of time, space, and ecology are involved here, and must be used in the analysis to elaborate on the existence of local races.

YELLOWSTONE LAKE

Yellowstone River originates atop the Continental Divide, draining to the north from Atlantic Creek and Two-ocean Pass. After flowing about 30 miles northward, the river enters Yellowstone Lake (fig. 1). The Yellowstone River drains from the north end of the lake and flows approximately 15 miles before reaching the Upper Falls of the Yellowstone, an impassable barrier 109 feet in height. Two-ocean Pass is also the origin of a branch of the Snake River, flowing to the west from Pacific Creek.

Yellowstone Lake lies at an altitude of 7,750 feet above sea level, and is 139 square miles in area. The lake is divided into several large arms and bays, and its irregular shoreline measures over 100 miles. Approximately 35 tributary streams enter the lake, and most of them support the spawning of cutthroat trout. The Yellowstone River below the lake is also used by trout for spawning.

The streams tributary to Yellowstone Lake are diverse in size, in temperature patterns, in flow, and in their chemistry. Many of them receive discharges from hot mineral springs, and high temperatures and pollution intolerable to cutthroat are present at some times in many streams. This diversity in the environment of the trout appears to have had some bearing on the formation of races within the lake and stream system. Variations in physical and chemical conditions also exist from place to place in the lake, and the distribution of groups of fish in the arms of the lake may be related to such environmental differences.

The Yellowstone cutthroat apparently came to Yellowstone Lake from the west, despite the fact that the lake lies east of the Continental Divide and drains into the Gulf of Mexico via the Yellowstone, Missouri, and Mississippi Rivers. Two-ocean Pass, mentioned above, lies astride the Continental Divide just south of Yellowstone Park. At times waters from the area flow into the Yellowstone and Snake drainages, and a continuous waterway is formed. Evermann (1893), after visiting the area, said, ". . . . . and there is no doubt whatever that trout can and do pass over this divide at will." He further stated, "Evidently Yellowstone Lake and the Upper Yellowstone River were stocked from the west, and almost certainly via Two-ocean Pass. The probability that the outlet of Yellowstone Lake at one time was toward the Pacific, as claimed by geologists, only strengthens this solution of the problem. But if this explains the origin of the trout of Yellowstone Lake, it leaves another equally interesting problem without any explanation, viz., the presence of the blob (Cottus bairdi punctulatus) in Pacific Creek and its absence from Atlantic Creek and the entire basin of Yellowstone Lake." Other theories have been advanced to explain the introduction of trout into this drainage, but, whatever route was used, the fish apparently have been established for a long time. Observers claim that trout today can pass over Two-ocean Pass, but no one holds that there is any considerable traffic across the top. The trout above the falls in the Yellowstone are, therefore, almost isolated in this drainage and represent the taxonomic entity Salmo clarki lewisi in its native waters.

LIFE HISTORY

The Yellowstone cutthroat in Yellowstone Lake is an adfluvial fish with a life history similar to those of several other inland cutthroats. Eggs are deposited in shallow redds in the gravels of streams tributary to the lake. Upon hatching, the fry may either move immediately downstream to the lake, may linger in the stream for a few months before descending to the lake, may spend the first winter in the stream, or may spend two or more winters in the stream. Most of

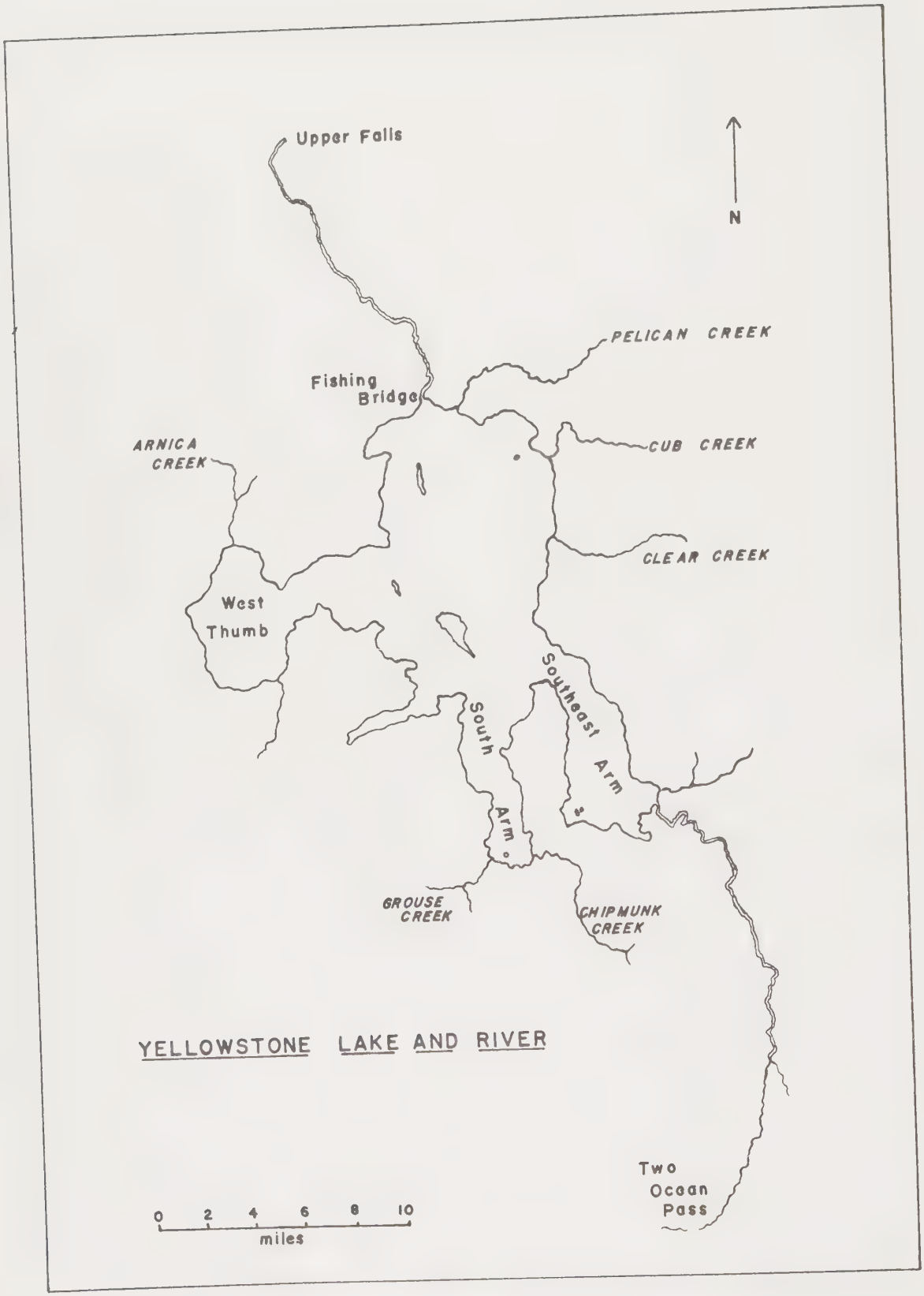

Figure 1.--Map showing the Yellowstone River drainage from Two-ocean Pass to the Upper Falls of the Yellowstone.

the immatures reaching the lake do so in their first season. Development to sexual maturity takes place in the lake, usually in three or four years. At spawning time, the adults ascend the tributary streams, the females dig the nests, spawning takes place, and the survivors of spawning and predation return to the lake. Of those remaining alive the following spring, some will again spawn that season, but a greater number will spawn the year following.

Careful measurements on these tributary streams have shown that mortalities in the egg stage are high, and usually less than one percent of the eggs laid will reach the lake as fry. Mortalities are low thereafter, but vigorous predation accounts for many adults, both before and after spawning.

Great distances are sometimes involved in migrations in the lake and in the Lower Yellowstone River, and considerable mixing occurs in the lake with fish from the several spawning streams. Segregation takes place at spawning time, and the spawners return to their natal streams for reproduction.

RACES IN SPAWNING STREAMS

Homing --The study of marked cutthroat at Yellowstone Lake has given an insight into the existence of races that are identified with spawning streams. The demonstration that homing is a strong urge in these populations permits us to recognize races that are distinct from each other geographically, and examination will later show that they are ecologically distinct, as well.

Spawners ascending Pelican, Chipmunk, Grouse, Arnica, and Clear Creeks were marked with Peterson disks in varying numbers. Fish in each of these streams were not marked each year, but from 1949 to 1953, 18,836 tags were applied. The fish were allowed to resume their upstream migrations and spawn, and the survivors were allowed to descend into the lake. After subsequent mortalities in the fishery and from natural causes, some were still alive at the next spawning time. Only 3.2 percent of returning spawners returned to streams other than those in which they were originally tagged. Table 1 summarizes the tag returns to spawning streams.

This overwhelming evidence may not be proof of what is called "homing", since the fish were not marked as immatures before they originally left their natal streams. It does constitute a basis, however, for the claim that each of these populations uses only its own stream, and can therefore be termed a local race. Homing in these fish has been discussed by Ball (1955).

The question may be raised concerning the lake habitat of these fish in support of the view that they may return to their own streams not because of their being part of a particular race, but because they spend their lake existence near the mouths of their own streams and use these streams because they are the closest ones at spawning time. This idea is partly refuted by more tagging evidence. Postspawners from some streams often travel considerable distances in the lake before returning to their streams for a second time. For example, tagged fish from Grouse and Chipmunk Creeks are taken in some numbers in West Thumb, as well as in the South Arm. Many of these are caught off the West Thumb Dock, a distance of 20 miles from the home streams. Fish from all streams are caught in greatest numbers in their own arms of the lake, but many are caught at distances. The distribution of angling pressure is an important point in these cases, affecting the localities of recovery of tagged fish. The evidence is clear that many fish return from great distances, and from other arms of the lake to spawn in their home streams. Tagging has also demonstrated that fish in the Yellowstone River ascend to the gravels near Fishing Bridge and spawn there, and that some fish from the lake move down to these same spawning beds at the same time. Each group then returns to the waters from which it started its spawning migration. Movements of 10 miles are common with these fish.

Another point of importance is that the postspawners from any stream are commonly caught in lake areas harboring fish from other streams. The lake fish are thus a mixture of fish from different streams, and at spawning time the various groups have equal opportunity to seek the closest stream for spawning. What happens, however, is that the groups segregate in the lake, and each race finds its own stream.

Table 1.--Returns of tagged fish to Lake Yellowstone streams, 1949 - 1955.

Stream	Number tagged	Total number returned in later spawning runs	Number to original stream	Number to other stream
Pelican Creek	6,043	74	71	3
Clear Creek	1,100	8	7	1
Chipmunk Creek	4,239	44	43	1
Grouse Creek	2,506	30	27	3
Arnica Creek	4,948	88	88	0
Totals	18,836	244	236	8

Time of migration--The migrational patterns of spawners entering tributaries exhibit differences from stream to stream. In some years spawning in all tributaries may be a week or two earlier or later than in the average year, but the patterns persist. Fishery workers at Yellowstone recognize "early" and "late" streams, and these temporal characteristics appear in remarkable fashion through the years. Their constancy is put to practical use in scheduling the installation of fish traps each year; it is commonly known, for instance, that the trap in Cub Creek need not be installed before June 1, because Cub Creek is a "late" stream.

Another feature of these migrational patterns pertains to the length of time of the run, and still another to the distribution of the run in relation to time. These principles are seen in figure 2, showing some time-of-spawning characteristics in five spawning streams. Each curve is typical of one stream, being based on five years' counts.

The five streams show five different patterns Pelican Creek has an early run which peaks in early June, has a second peak in late June, and lasts until late July. Chipmunk Creek begins early (the curve suggests that a portion of the run may enter the stream even before the trap is installed) and extends to the end of July. These runs show a very definite bimodal feature, with both peaks at about the same level. Grouse Creek, located close to Chipmunk Creek in the South Arm, shows the bimodal quality, but with a definite emphasis later in the season. Grouse Creek starts later than Chipmunk, ends at about the same time, but has its second peak dominating the distribution. Clear Creek, essentially a late run stream, has a suggestion of a mode in early June, but by far the greatest portion of the migration occurs in the middle of July. Cub Creek shows one great, late surge in early and middle July, and rapidly diminishes in late July.

These migration characteristics, occurring in fairly constant patterns every year, appear to be, in at least some cases, responses to temperature characteristics in the streams. Arnica Creek, an early run stream, ordinarily has maximum daily water temperatures in early June well above 50° F. Grouse Creek, a late stream, averages close to 45° at this time, while Chipmunk and Pelican Creeks average about 50°. The notable point here is that, if times of migration are partly responses to temperatures, only the fish related to specific streams respond and enter these streams. The lake contains a mixture of fish from several streams, many having the opportunity to take part in each migration as it occurs. But, each group segregates from the mixture at the proper time and enters its own stream.

Sizes of fish--The recognition of races of fish is often accomplished by demonstrating statistically significant differences in sizes of fish. The differences are presumably due to differences either in growth rate or in age composition. Such differences have been reported for cutthroat trout in spawning runs and in the

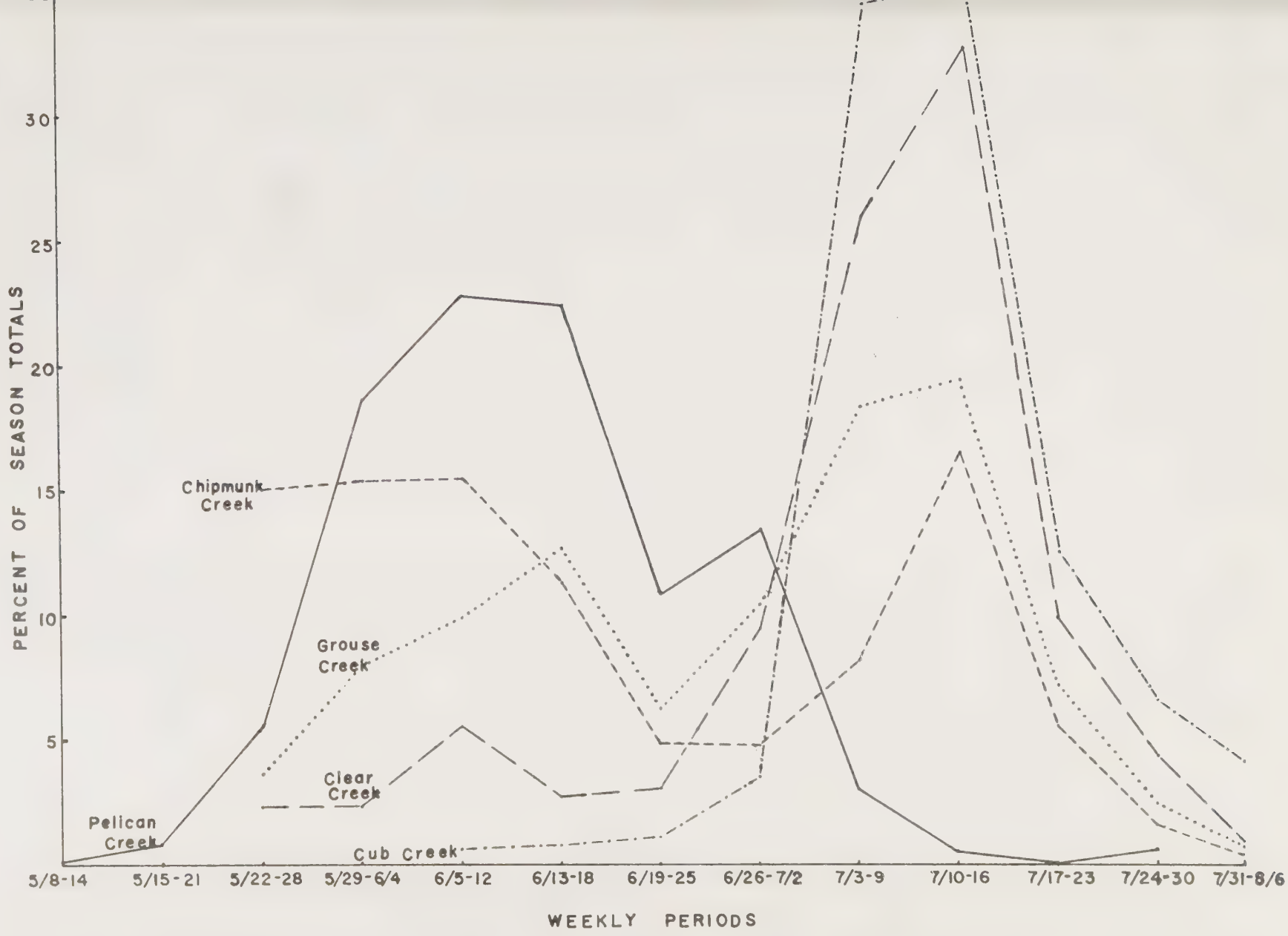

Figure 2.--Distribution throughout the season of percentage of total season spawners for five Yellowstone Lake tributaries, based on counts of five seasons.

fishery at Yellowstone (Cope, 1953). These length data were analyzed without regard for ages of the fish. If we can assume that year-class composition remains fairly constant in the spawning runs from stream to stream, we see that som e streams have populations which differ significantly from those of other streams. The 1952 spawning runs into Grouse Creek averaged 374.7 millimeters in mean total length, which is significantly different from the spawners of the Yellowstone River, measuring 367.7 millimeters, those of Pelican Creek, measuring 355.9 and of Arnica Creek, measuring 353.5. Chipmunk Creek characteristically supports the largest spawners of all these streams, and in the years when sampling was good the size superiority was statistically significant.

If racial differences in sizes cannot be claimed for these spawning populations because lengths of fish of like year classes were not compared, then a claim may be made on the basis of differences in age composition of the spawning runs. The fact that the size differences appear from year to year, with the different streams retaining their same relative ranks, suggests that variation in year-class composition may be fairly small from year to year, and that the differences noted above may be related to racial differences in this drainage. Figure 3 illustrates the extent of differences in length for three streams throughout the 1945 season. The same general relationship between these three streams was repeated in 1951, 1952, 1953, and 1954.

The dispersion about the points which control the curves in figure 3 can be visualized from the following coefficients of variation: May 28-29; Chipmunk Creek, 4.09; Grouse Creek, 5.23; Pelican Creek, 7.78. July 2-4; Chipmunk Creek, 5.79; Grouse Creek, 6.47; Pelican Creek, 8.61. July 11-14; Chipmunk Creek, 5.37; Grouse Creek, 5.56; Pelican Creek, 6.90. The sizes of fish at the beginning of the spawning runs are significantly different, from stream to stream, but the significance is lost as the season progresses. The mean lengths of fish in all streams becomes smaller with the progress of the season.

The influence of differential fishing pressures on age and size composition of spawning runs must be considered here. If trout from certain streams are subjected to unusually heavy fishing pressures which take high tolls of the larger and older fish, subsequent spawning runs will probably contain smaller fish. This has been suggested (Cope, 1953) for Yellowstone Lake, where heavy fishing pressures in the north end of the lake appear to have affected the sizes of spawners in Pelican Creek and of fish taken in the Fishing Bridge area fishery. Chipmunk and Grouse Creeks do not seem to have been affected to such an extent. Fish size still appears to relate to racial differences, however, because size differences were measured in the years before fishing pressure became so heavy. Also, compare Chipmunk Creek fish with those of Grouse Creek. The two streams enter the South Arm not far from each other, the postspawners occupy the same parts of the lake and are subject to the same fishing pressure, the populations are usually about the same size, and yet Chipmunk Creek spawners are consistently larger than those of Grouse Creek.

Size differences have also been demonstrated in fish taken from two parts of the lake fishery, the West Thumb and the Fishing Bridge areas. Here we are dealing not with individual races, but with mixtures of races. Fish taken in the Fishing Bridge area fishery (in the northeast part of the lake) are consistently larger than those caught in West Thumb, at the beingning of the season and thereafter. Tagging has shown that fish from certain streams commonly move to certain parts of the lake after spawning, even though the preferred lake habitat may be some distance from the spawning stream. Despite the fact that straying into other portions of the lake occurs to a minor extent, the lake populations in each part of the lake are dominated by fish from the same streams each year. There are, then, certain races of fish associated with particular lake areas, as well as with particular spawning streams, and this holds true from year to year.

Sizes of eggs.--There is not a great deal of morphometric information available from cutthroat in Yellowstone Lake, but there are records pertaining to egg size and numbers that seem to show that fish from different streams bear eggs of different sizes and in different numbers. Keeping in mind the general principle that the

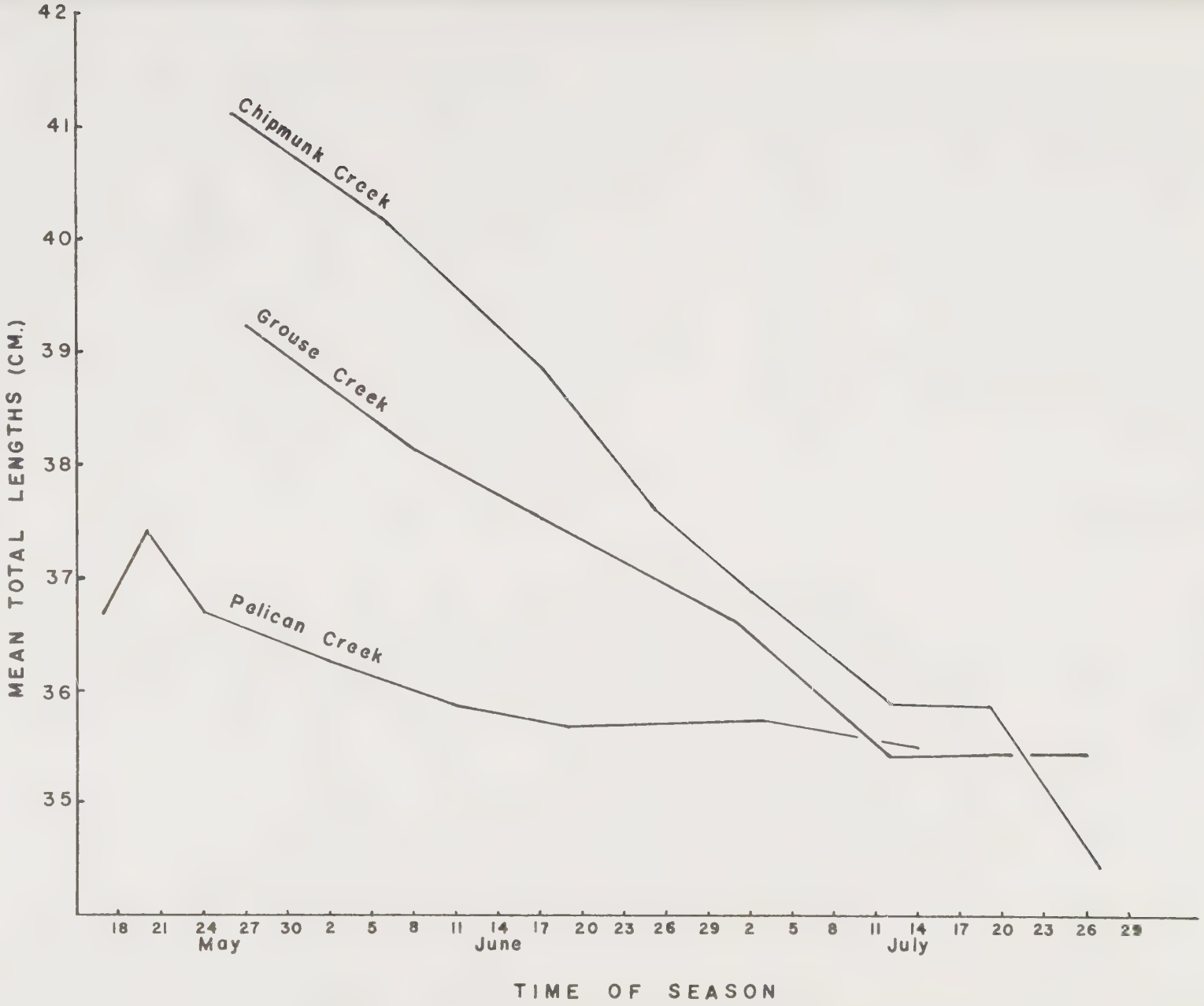

Figure 3.--Distribution throughout the 1945 spawning season of mean total lengths of spawners in three tributaries of Yellowstone Lake.

larger trout produce more eggs and larger eggs than do the smaller trout, we perceive differences in eggs that are independent of the differences in fish size between streams.

Table 2 is based on Yellowstone hatchery egg-taking records for the years 1941 through 1952. We do not have length data for all streams for comparison with the numbers in table 2, but available measurements indicate that Chipmunk Creek fish average among the largest, Pelican and Arnica Creek fish are small, and Grouse Creek fish are intermediate in size. If fish of these streams were all of the same race, we would then expect Chipmunk Creek females to have the largest eggs and the greatest number per female, for Grouse Creek to be intermediate in these relationships, and for Pelican Creek and Arnica Creek females to have small eggs and small numbers per female. The table shows that Chipmunk Creek does, indeed, have the largest eggs (262 per ounce) and Pelican Creek the smallest (307 per ounce), and that Grouse Creek occupies an intermediate position (286 per ounce). Arnica Creek, however, with 268 eggs per ounce, has the second largest eggs on the list, rather than having the small eggs that the fish size would lead us to expect. Chipmunk Creek has a relatively small number of eggs per female (981) instead of the large number we would expect. Pelican and Arnica Creeks, with 979 and 899, respectively, have the low numbers we would expect (but with rather large discrepancy between them), and Grouse Creek has the expected intermediate number (1,007). We are faced with the fact that we do not have length measurements in these streams for all the years from 1941 through 1952, but measurements are available for several of these years, and the relative sizes of fish in these streams have been very consistent through these years.

Figure 4 plots regressions of total numbers of eggs on ovary weight for three streams. These relationships are based on measurements made by Dr. Stillman Wright in Pelican, Chipmunk, and Grouse Creeks in 1945. The trends show that Pelican Creek fish have more eggs per ovary weight than those of Chipmunk Creek, and Chipmunk Creek fish more than those of Grouse Creek. This is evidently not related to size of fish, since Chipmunk Creek fish are larger than are Grouse Creek fish.

Coefficients of regression were calculated for each of the regressions in figure 4, and then compared according to the method described by Simpson and Roe (1939), page 279. These tests, for significance of differences between regression coefficients, resulted in the following t values: Grouse Creek vs. Pelican Creek - 17.207; Grouse Creek vs. Chipmunk Creek - 8.774; Chipmunk Creek vs. Pelican Creek - 8.166. These values indicate that the relationship for each stream is very significantly different from that of each of the other two streams.

These relationships involving eggs appear to show that the fish of certain streams have specific characteristics that are different from those of adjacent streams as well as remote streams.

SUMMARY

Yellowstone Lake and the Upper Yellowstone River, and possibly part of the Upper Snake River drainage, form a closed system. The Yellowstone cutthroat is held within these waters, and no introduction has been made within the history of fishery work. These fish are thus suited to studies on races. Several spawning tributaries to Yellowstone Lake and the river have been studied to determine the existence of distinct races in this drainage.

The association of groups of fish with particular spawning streams has been established through tagging. Homing to streams occurs in 97 percent of the spawners, suggesting that each stream has its own race of trout. Migrational patterns in the lake after spawning are quite constant from year to year, races from certain streams often moving great distances. Mixing of races takes place in the lake, and each part of the lake appears to contain about the same mixture each year.

Times of migration into five streams were examined for a five-year period, and five different patterns were perceived. The patterns were very constant from year to year, some streams supporting early runs, some late runs, some having bimodal distributions, and

Table 2.--Some characteristics of cutthroat eggs taken for the Yellowstone hatchery, 1941 through 1952

Stream	Total eggs taken	Total number of females	Total weight of eggs (ounces)	No. eggs per female	No. of ounces per female	Number of eggs per ounce
Pelican Cr.	53,125,206	54,292	173,135	979	3.189	307
Cub Cr.	9,406,065	9,479	33,805	992	3.566	278
Clear Cr.	39,609,160	38,917	144,788	1,018	3.720	274
Columbine Cr.	18,580,614	18,948	66,890	981	3.530	278
Chipmunk Cr.	54,298,802	55,368	206,853	981	3.736	262
Grouse Cr.	36,990,787	36,726	129,305	1,007	3.521	286
Hatchery Cr.	4,263,388	4,111	14,855	1,037	3.613	287
Arnica Cr.1/	4,599,998	5,116	17,185	899	3.359	268

1/ These records cover the years 1936 through 1940

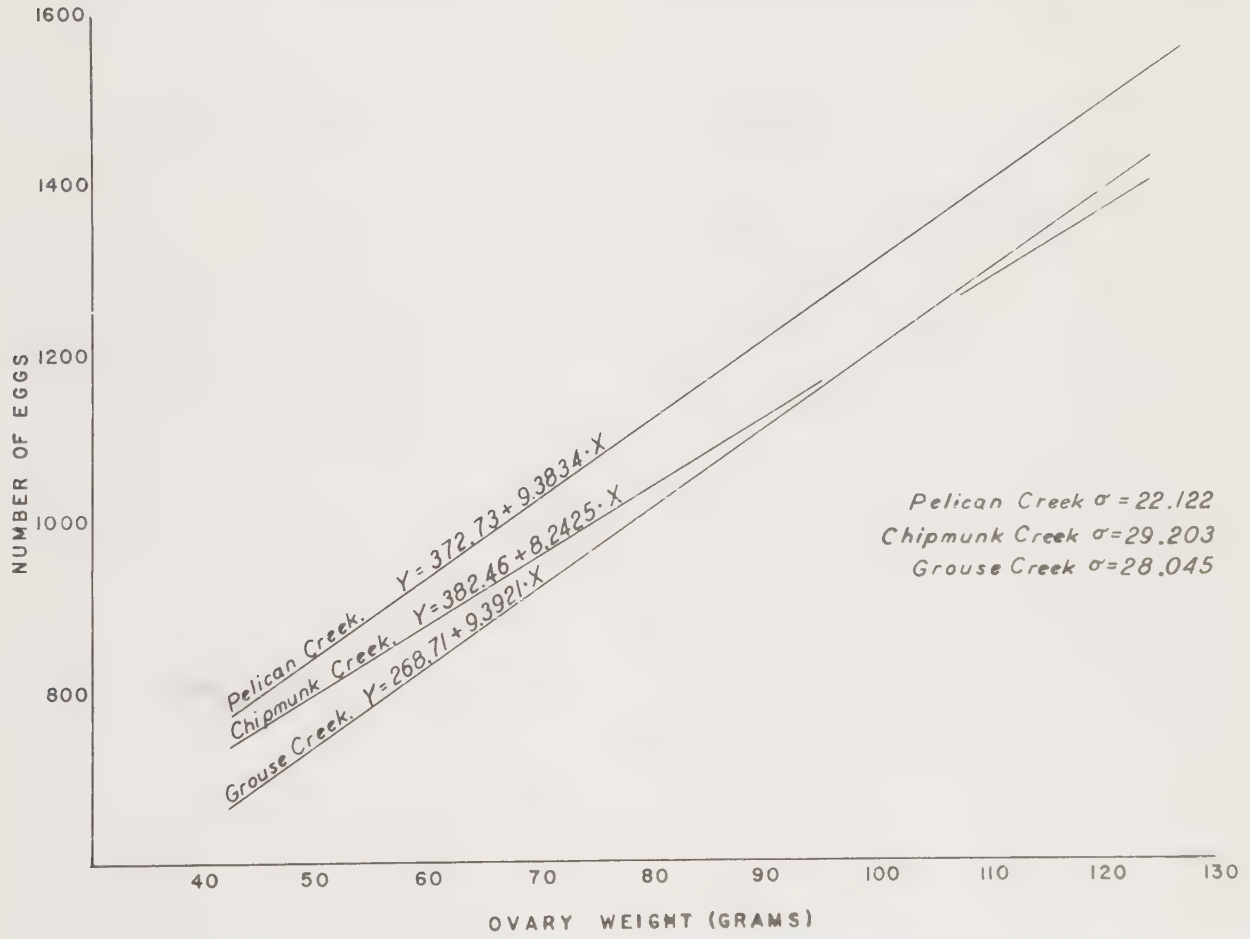

Figure 4.--Regressions of numbers of eggs on ovary weight for females in the 1945 spawning runs of three Yellowstone Lake tributaries.

some having essentially a single mode. Water temperature records were examined in connection with times of migration, and there appears to be a relation between the two. Of the fish in the lake, however, only those of a certain race will segregate and respond to the stream temperatures of its own stream. These races might, then, be geographical races, ecological races, or temperature races.

Differences in mean total length of fish in spawning runs have been measured, and significant differences found between streams The differences may be due to differences either in age composition or in growth rate, but in either case they would be racial differences, since sizes of fish in different streams bear the same relationship to each other year after year. Size differences have also been compared for fish caught in two areas of the lake, and have been found to be significantly different.

Sizes of eggs and numbers of eggs per female from eight streams were compared in relation to sizes of fish. Counts and measurements did not always correspond to the figures that would be expected if the fish were all of the same race. Some streams had small fish with relatively large eggs, suggesting that some races diverged from the general relationships to be expected. Difference in numbers of eggs per unit of ovary weight were measured among three streams.

Acknowledgment is made of the data on egg numbers and ovary weight collected by Dr. Stillman Wright, of the records on hatchery egg take provided by Mr. William Dunn, Superintendent of the Yellowstone hatchery, and of the fish counts and measurements secured by many fish culturists and biologists of the U.S. Fish and Wildlife Service. Mr. Martin Laakso, Fishery Research Biologist at Logan, very kindly aided in checking computations.

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SUBPOPULATIONS OF THE STRIPED BASS ROCCUS SAXATILIS (WALBAUM), IN TRIBUTARIES OF CHESAPEAKE BAY[1/]

By

Edward C. Raney[2/]

INTRODUCTION

The striped bass, Roccus saxatilis (Walbaum), is widely distributed along the Atlantic coast from the St. Lawrence River to the northern part of the St. Johns River, Florida, and in the Gulf of Mexico from west Florida to tributaries of Lake Pontchartrain, Louisiana. Throughout its range it spawns in fresh water. In southern tributaries of Chesapeake Bay, Tresselt (1952) reported that its eggs were deposited in fresh water usually within 25 miles of brackish water. The time of spawning varies with latitude and local temperature conditions; in the tributaries of Chesapeake Bay most spawning occurs in late April and May.

In the Gulf of Mexico and at both extremes of the range along the Atlantic Coast it is more of a freshwater species and rarely undertakes coastal migrations. In southeastern United States it is known to move upstream as much as 100 miles to spawn and at least one population, that of the Santee-Cooper reservoirs of South Carolina, has been shown by Scruggs and Fuller (1955) to be maintained at a high level without returning to brackish or salt water. The biology of the striped bass has been reviewed by Raney (1952).

The largest numbers of striped bass are found in Chesapeake Bay and its many large tributaries. The classic studies of Merriman (1937 and 1941) demonstrated that the bulk of the migrating schools which pass northward along the coast in spring and southward in the fall was largely of Chesapeake Bay origin. An immense year class was produced in the Chesapeake Bay area in 1934 from one of the smallest spawning populations then on record. This year class produced large catches in Chesapeake Bay and coastal fishery in 1936 and 1937. Fortunately, this was followed by frequent successful year classes and the present numbers of striped bass remain high. Other populations are important locally like that found in the Hudson River; this population is a separate race (Raney and de Sylva, 1953) and has a different migratory pattern (Raney, Woolcott, and Mehring, 1954).

Optimum conditions for striped bass production exist within the Chesapeake Bay region. The many large rivers serve as breeding and nursery grounds and Chesapeake Bay, with an abundance of forage fishes, is an excellent feeding area for juveniles and adults. Tagging experiments by Pearson (1938) and tagging and meristic studies by Vladykov and Wallace (1938 and 1952) suggested that different subpopulations exist within the Chesapeake Bay region. Raney and de Sylva (1953) produced further evidence, based on relatively few specimens, of subpopulation differences in the Chesapeake Bay area. Studies of numerous samples of young of several additional year classes from most of the large and productively important Bay tributaries have shown differences which are interpreted as indicating the presence of several subpopulations within the Bay. The results reported here are based largely on fin ray counts. Where morphological differences have not yet been shown, the

1/ This study was supported by the U.S. Fish and Wildlife Service as part of the Atlantic States Cooperative Striped Bass Program of the Atlantic States Marine Fisheries Commission for which the author serves as coordinator. William S. Woolcott, who assisted in this study when a graduate student at Cornell University, was a recipient of a Sport Fishing Institute Fellowship Award, 1953-54.

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assumption is made that the populations are the same, although it is quite clear that future studies of other characters may show differences. Studies involving pectoral ray numbers, the number and characteristics of scales, numbers of gill rakers, and measurements of body parts are under way on samples taken in Chesapeake Bay and elsewhere as part of the Atlantic Coast Cooperative Striped Bass Program.

This and earlier studies were made possible by the cooperation of several agencies and many fishery biologists. William H. Massmann of the Virginia Fisheries Laboratory furnished specimens from the York River system that stimulated the study by Raney and de Sylva (1953) which suggested that different races exist in the Hudson River and Chesapeake Bay. He has continued to help and has actively participated in the field work in Virginia. The use of the vessel "Virginia Lee" which was made available by Director J.L. McHugh of the Virginia Fisheries laboratory is gratefully acknowledged. C. E. Richards and Jesse Hobbs of the Laboratory assisted with the collecting during July, 1954 and 1955. In Maryland the cooperation of Edgar H. Hollis and Harold A. Davis, Jr. of the Department of Tidewater Fisheries made available the bulk of the specimens studied from tributaries of the upper Bay. L. Eugene Cronin, Romeo Mansueti, Richard E. Tiller, and Earl T. Walker of the Maryland Department of Research and Education, Solomons, have made special efforts to get material from the Patuxent and Potomac rivers. Through the interest of John R. Greeley and W. Mason Lawrence of the New York State Conservation Department, support was received for field work on the Hudson River during 1954. Warren F. Rathjen and Lewis C. Miller assisted with field work and made available collections taken during 1954. Cornell graduate students Earl E. Deubler, Jr., C. Richard Robins and William S. Woolcott assisted during several field expeditions and all counts taken on specimens obtained in 1953 and 1954 were made by the latter. Charles F. Cole made the counts on most of the 1955 material. Robert Minturn Lewis made some of the statistical calculations but the chi-square tests were run at the Cornell Computing Center through the courtesy of its Director, Richard Lesser. Advice on statistical methods has been received from Douglas S. Robson and Robert G. D. Steel of Cornell University, James R. Westman and Kenneth W. Meinken of Rutgers University, and J. L. McHugh of the Virginia Fisheries Laboratory. Helpful suggestions for the improvement of the manuscript were made by Gerald B. Talbot of the U. S. Fish and Wildlife Service, Donald P. de Sylva of the University of Miami Marine Laboratory, William E. Fahy and Earl E. Deubler, Jr. of the University of North Carolina Institute of Fisheries Research, and John R. Greeley of the New York State Conservation Department.

The terms species, subspecies, and race seem adequate to describe the major levels of differentiation found in the striped bass. As here used, race implies a lesser rank than subspecies. The definitions of the terms population and subpopulation as proposed by Marr (first paper in this group) are followed.

There seems to be only one species of striped bass. Its closest relative, the white bass, Roccus chrysops (Rafinesque), inhabits fresh water in the Mississippi River and Great Lakes drainages. The population of striped bass found in tributaries of the Gulf of Mexico is 100 percent differentiated in number of lateral line scales from the nearest Atlantic coastal population of the St. Johns River, northeastern Florida. The lateral line scales in 11 specimens available from tributaries of the Gulf of Mexico range from 62 to 68, mean 65.91 (S.D. 1.97, S.E.0.59), and in the St. Johns River sample of 42 specimens, they range from 52 to 58, mean 54.33 (S.D. 1.44, S.E. 0.22). Data for lateral-line scale counts were given for samples from other southeastern localities by Raney and Woolcott (1955: 446). Perhaps there has been no exchange between the St. Johns River and Gulf of Mexico populations since the emergence of the Florida peninsula because the tropical waters of southern Florida serve as a barrier.

Along the Atlantic Coast the apparent lack of coastal migration in the region of southern North Carolina to northeastern Florida has made free gene exchange unlikely. Two races present in the southeast which were designated by Raney and Woolcott (1955: 449) as the St. Johns River race and the Santee-Cooper race. In the small sample from the Coastal Plain of South Carolina characters are intermediate as would be expected

in the case of intergradation between infraspecific categories. The characters of the populations of the rivers of eastern Georgia are as yet unknown.

The two races which have been studied most are those of the Hudson River and Chesapeake Bay. Tagging studies by Merriman (1941: 44) and Vladykov and Wallace (1952: 172) indicate little exchange between the striped bass populations in Chesapeake Bay and Albemarle and Pamlico Sounds, North Carolina. Some meristic data also indicate that they differ; the latter are separable from their nearest neighboring population in the James River on the basis of dorsal fin spines. Final decision on the status of this population is deferred until the completion of studies now under way.

MATERIALS

This study is based entirely on young striped bass which are those in their first summer or fall (to January 1). It seems certain that these young were spawned in the river in which they were found. The pelagic larvae are swept back and forth by tidal currents. When they are approximately 13 to 19 mm. in standard length the young are first found in small schools over the beaches. Counts were made on young which were 35 mm. or more in standard length; all had the definitive number of fin rays (table 1).[3/] It is assumed that no sexual dimorphism exists for the characters enumerated.

Series were collected expressly for this study. Most were taken from July through October and an attempt was made to get material from a wide geographic range in each river. When possible, collections were made at different times at each locality and some composite samples, especially those from the Hudson River, included as many as 10 samples. Samples were taken with seines, the most effective of which was a 25 foot by 5 foot knotted bag seine with one-fourth inch bar mesh. Various shore habitats were sampled over a period of several years but the only types to yield satisfactory numbers were sand, or sand-gravel beaches located on points which were swept by tidal currents; 25 to 100 young were often collected in a single seine haul. Few young were taken by trawling off shore near such points although yearling striped bass were found. Adjacent coves with silt bottom, with or without aquatic vegetation, were seined but yielded few striped bass. The quiet cove was the favored habitat of the related white perch, Roccus americanus (Gmelin). Young striped bass were often associated with young American shad, Alosa sapidissima (Wilson), while white perch were frequently found with the glut herring, Alosa aestivalis (Mitchill).

3/ The tables appear at the end of this paper.

METHODS

Counts were made of spines in the first dorsal fin, and of the soft rays in the second dorsal and anal fins. In counting the dorsal and anal soft rays, the method of Hubbs and Lagler (1949: 10) was followed; the last two fin-ray bases were counted as one. All dorsal spines were enumerated. Because three persons counted it is possible that this is a source of bias despite the effort to assure agreement on methods. All dorsal spine counts were rechecked by the author.

Because the distributions of each of the three characters are primarily binomial the chi-square test was used (Snedecor, 1946). In setting up the data in tables 2-22 for this test, those data for the numbers of first dorsal fin spines were lumped as 9 or fewer vs. 10 or more; for soft rays in the second dorsal, as 11 or fewer vs. 12 or more; for anal soft rays, as 10 or fewer vs. 11 or more; for the character index, as 13 or fewer vs. 14 or more. It may be of interest to report that the analysis of variance and the t-test gave the same indication of significance and at the same level in every case except for the data in table 18 where the F-test gave an indication of difference at the 5 percent level whereas the chi-square indicates a difference at the 1 percent level. The results of the tests (X^2 and t values) are included in the tables; the abbreviation N.S. indicates that there was no significance at the 5 percent level.

A meristic or character index is used in studying correlated characters (tables 21 and 22). The computations (adding and subtracting) are performed for the data on each specimen and frequency distributions are then constructed. Some use has been made of the "percentage of

separation" (and intergradation) of Ginsburg (1938).

In all tables the upstream localities are at the top, and all east shore tributaries of Chesapeake Bay are followed by an asterisk. The term "Upper Bay" refers to the region north of the Rappahannock River. Data for the James River include stations within its large tributary, the Chickahominy River. In tables listing James River samples the number following the localities such as J29 and C43 indicate the distance in nautical miles from the mouth of the James River.

RESULTS

Spines in First Dorsal Fin--The dorsal fins are separate. The membrane attached to the posteriormost spine of the first dorsal fin terminates on the dorsum well in front of the origin of the second dorsal fin. On gross examination all ray elements in the first dorsal fin seem to be spines, viz., single, median, unsegmented, sharp, and hardened structures. It does not appear likely that an increase in number of spines in the first dorsal fin is caused by a shifting forward of a spine from the second dorsal fin because the latter invariably contains one spine which is situated anteriorly.

The counts were made using adequate magnification. The most anterior spine may be small and sometimes is close to the next best developed spine; however, no attempt was made to dig into the skin or muscle at the origin of the dorsal fin. It seems likely that this small spine might easily be overlooked in larger juveniles or adults, and it is probable that it may regularly become buried in the flesh. The posteriormost spine in the first dorsal fin is normally not much smaller than the penultimate spine but is more recumbent. It is easily sighted when the anterior spines are pulled upright.

In order to determine if there is an increase in number of first dorsal spines with size, the standard length of young specimens with 9 or fewer dorsal spines was compared with those having 10 or more which were taken in the James River in 1954 (table 1). The mean values are not statistically different.

Throughout the range of the striped bass the modal number of spines in the first dorsal fin is 9. The range is 7 to 12 with 10 being the second most frequent count; 8 and 11 spines are counted infrequently and 7 and 12 occur rarely. When a shift occurs it is from 9 toward 10 rays. Therefore those with a higher mean value generally have a greater variance.

In the earlier studies of races in striped bass by Raney, et al (1953, 54, 55), the number of first dorsal spines seemed of little value. However, after the numerous series of young obtained in the summers of 1954 and 1955 from many tributaries of Chesapeake Bay were counted it was noted that those from the James River system averaged high and a check of this character in other Atlantic Coast samples showed this to be an unusual count which was approached only by samples from the Hudson River.

The first dorsal spine number is rather constant within the James and Hudson, the only two rivers where adequate samples were available from several localities. The frequency distributions of samples taken in July, 1955 from six localities in the James River are given in table 2. The extreme localities are separated geographically by a distance of approximately 30 nautical miles. No trend is noted for the mean values and chi-square shows no significant difference at the 5 percent probability level and indicates that all samples could have been drawn from the same population. A composite sample from two upstream localities in the James River is compared with that from four downstream localities (table 3); a test indicates that the two samples are not significantly different at the 5 percent level. An analysis for spine counts of young taken on July 23 and 24, 1954 (table 4) gave similar results. A high dorsal spine count was obtained from a sample taken in the Chickahominy River at Shackleford Farm near Lanexa in 1954 (table 5) was compared with a down-river sample; a test indicates a statistically significant difference. This upstream high count may represent an unusual sample but in any event it is different in the direction in which the James sample is specialized. A sample of 21 young taken at Shackleford Farm on July 26, 1949, had a mean value of 9.19 which is more nearly in line with expected values.

The number of dorsal spines in the numerous samples of young taken in the Hudson River in 1954 also gives a picture of relative constancy. The sample from each locality on the Hudson River (table 6) is composite and represents varying numbers and sizes of subsamples taken mostly from mid-July to October. The maximum distance between the upstream sample taken at Coxsackie and the downstream sample from Palisades is approximately 100 miles. No trends in mean values are observed. A test indicates that the samples are not significantly different at the 5 percent probability level and all samples could have been drawn from the same population. The lower Hudson River from the Palisades to Peekskill is much wider and more saline than the reaches above Beacon. When the same data are compared as up- vs. downstream samples (table 7), the means are identical and a test of the samples indicates that there is no significant difference. The means of up- and downstream samples of young taken in 1953 (table 8) are close and the difference is not significant.

Thus it has been shown that no statistically significant differences occur in up- vs. downstream samples of young of the 1954 and 1955 year classes from the James River nor for the 1953 and 1954 year classes from the Hudson River.

The difference in mean values for the first dorsal spines between the James River sample and those taken elsewhere along the coast, especially with those of other Chesapeake Bay tributaries, is noteworthy. The data (tables 9-10) for samples of young taken in 1955 and 1954 from tributaries of Chesapeake Bay disclose high values for both year classes from the James River. There is consistency within the system. The Chickahominy River considered alone has a high mean value of 9.30 (27 specimens) in 1955 and 9.49 (61 specimens) in 1954.

A test of the 1955 samples (table 9) gives a significant difference at the 1 percent level and indicates they very probably were not all drawn from the same population. Because an inspection of the mean values indicates that the James sample is outstandingly different, it was tested against that of the composite sample from the Elk to the York (table 9); $X^2 = 50.66$ which is significant at the 1 percent level and indicates that the two very probably were not drawn from the same population.

The same indication of heterogeneity was found for 1954 samples from Chesapeake Bay tributaries (table 10) and again the James sample is obviously different. When tested against all the rest (Choptank to York in table 10) $X^2 = 112.78$, which is significant at the 1 percent level and indicates that the two samples very probably were not drawn from the same population.

In order to compare the dorsal spine values of East Shore tributaries, West Shore tributaries (less the James River system), and the James River, the samples were regrouped in table 11. A test of these composite samples indicated a significant difference at the 1 percent level. The East and West Shore means are close and are obviously different from the James sample.

The data on dorsal spines for Chesapeake Bay tributaries are arranged in geographic blocks from north to south (table 12). A test gives a value which is significant at the one percent level and which indicates heterogeneity. It is obvious from an examination of the mean values that the James River sample differs most markedly from the other three composite samples.

On the basis of the dorsal spine character it is concluded that in the Chesapeake Bay area there are two subpopulations. The James River subpopulation is differentiated from all others studied within and outside Chesapeake Bay.

Samples from the Hudson River have the next highest count; when the data for samples of young of the 1954 year class are compared with those from the James River (table 13) a test shows the difference to be statistically significant and the population to be heterogeneous.

Soft Rays in the Second Dorsal Fin--The modal number in samples studied throughout the range of the striped bass is 12 rays except for those from the Hudson River where it is 11 except in very few samples. The range is from 9 to 14 but counts of other than 12 or 11 are un-

usual. In young specimens taken in the James River in 1954 there is no significant difference in the mean values for standard length of the sample which had 11 or fewer rays, and that which had 12 or more rays (table 1).

When the frequency distributions of dorsal soft rays of the several samples taken in the James River in 1955 (table 2) were tested, homogeneity is indicated. The composite upstream sample for 1955 averaged slightly lower than the downstream composite sample (table 3) but when tested the results indicate homogeneity.

The distribution of soft dorsal fin rays in samples of young taken in the James River in 1954 are given in table 14. A test indicates that all samples could have been drawn from the same population. However, the downstream samples are somewhat higher in mean value; when up- and downstream composite samples (table 4) are tested there is a significant difference at the 5 percent level which indicates that they probably were not drawn from the same population. A comparison of an upstream and a downstream sample of young of the 1954 year class from the Chickahominy River, a large tributary of the James River, is shown in table 5, but, although the downstream sample averages higher, a test shows no heterogeneity.

The frequency distributions of dorsal soft rays in samples of young taken in the Hudson River in 1954 are given in table 15. A test indicates that they very probably were not drawn from the same population. An examination of the mean values (table 15) shows that those from downstream localities average higher. When the composite upstream sample is compared to that from downstream (table 7) a test indicates difference at the one percent level. Similar results were obtained from the 1953 year class when an upstream sample (Coxsackie) was compared with one from downstream (Haverstraw) (table 8). The downstream sample has a higher value which is significantly different at the one percent level.

In summary it is noted that the number of soft rays in the second dorsal fin is slightly, but not always significantly, higher in the downstream samples from the James River. The Hudson River consistently has higher values for downstream samples. Here a much greater distance is involved than in the James River. The higher downstream values perhaps are correlated with differences in ecology since the lower Hudson River contrasts with the upper river in several respects as noted above.

The frequency distributions of the number of soft dorsal rays in samples taken from the tributaries of Chesapeake Bay are given in tables 16 and 17. When the eight samples taken from tributaries of Chesapeake Bay in 1955 are compared (table 16) the low value for the James sample especially when compared with the adjacent York-Rappahannock samples is noteworthy. When these two (James vs. York-Rappahannock) are tested, $X^2 = 98.04$ which is significant at the one percent level and indicates that they very probably were not drawn from the same population. When the James sample is compared with the composite Elk to Wicomico sample (table 16), $X^2 = 17.49$ which is significant at the one percent level and indicates probable heterogeneity. The data for samples of the 1954 year class (table 17) shows the same general trend for these three samples. When the James and York-Rappahannock samples (table 17) are tested $X^2 = 63.51$ and when the James is tested against the composite Choptank-Pocomoke sample $X^2 = 24.45$; both of these results are significant at the one percent level and indicate heterogeneity.

The composite 1954-55 samples comparing East Shore tributaries, West Shore tributaries (excluding the James River), and the James River are given in table 11. The value for the James River sample is obviously different and a test indicates that the three very probably were not drawn from the same population.

The frequency distributions representing composite 1954-55 samples (table 12) are arranged in four geographic groups from north to south. A test indicates that they very probably were not drawn from the same population. The Elk to Chester and Choptank to Potomac-Pocomoke composite samples have identical means and seem to represent a population which differs from the Rappahannock-York sample and the James River sample. Although the Pocomoke sample is included with the Choptank to Potomac sample in the geographic arrangement, the data (table 17) suggests that it might belong with the Rappahannock system.

An examination of the mean values for dorsal soft rays and spines (table 12) shows that the latter is high and the dorsal soft ray count is low in the James sample. The reverse is true of the York-Rappahannock sample and the composite upper Bay sample is intermediate for each count.

On the basis of dorsal ray counts and with due consideration for the differences in environmental conditions which may occur at the same locality from year to year and the slight increase in mean values noted for some downriver samples, there seems to be three subpopulations represented within Chesapeake Bay, namely Upper Bay, York-Rappahannock, and James.

A comparison of soft dorsal fin rays in samples from the Hudson and James Rivers is given in table 13. A test shows the difference to be highly significant at the one percent level and indicates they very probably were not drawn from the same population. Incidentally, the mean values for these two river systems are the lowest yet encountered for this character.

Soft Rays in the Anal Fin--The number of anal spines is almost invariably three in young specimens more than 25 mm. in standard length; during development up to this size the third spine is derived from a soft ray. Throughout the range of the striped bass the modal number of anal soft rays is 11. The distributions range from 7 to 13 rays, but 10 is the only other number which occurs frequently. When the mean standard length of young specimens with 10 or fewer rays was compared with that of specimens having 11 or more rays (table 1) taken in the James River in 1954, no statistically significant difference was obtained.

The frequency distributions of anal soft rays in samples taken in the James River in 1955 are given in table 2. A test shows the differences to be not significant and indicates that the samples could have been drawn from the same population. With the same data arranged in up- vs. downstream composite samples (table 3), there is only a slight difference in the mean values which is shown by test to be not significant. When tested the samples of the 1954 year class from the James River (table 14) show no significant difference which indicates the samples could have been taken from the same population. When these data are considered as up- and downstream composite samples (table 4), the slight differences in values are shown to be not significant. Up- and downstream localities in the Chickahominy River are compared (table 5) and although there is a slightly greater downstream value it is not statistically significant. Thus for anal soft ray counts within the James River for the two year classes studied no significant increase was noted for downstream samples.

The distributions for samples of anal soft rays for the 1954 year class from the Hudson River are given in table 18. A test indicates that the samples very probably were not drawn from the same population. When the data are arranged in up- vs. downstream composite samples (table 7), the mean value of the downstream sample is higher and a test indicates a significant difference which indicates that the two samples very probably were not drawn from the same population. The data for up- and downstream samples of young taken in 1953 (table 8) give the same result.

In summary it is noted that the values for downstream samples in the Hudson are significantly higher than for upstream samples; this trend is like that exhibited by second dorsal soft rays. The slight differences again may be due to average differences in environmental conditions.

The data for anal soft rays in samples taken from several tributaries of Chesapeake Bay in 1955 and 1954 (tables 19 and 20) exhibit moderate differences in mean values for different year classes. However, even the greatest differences which are found in the Nanticoke ($t = 0.976$) and the Wicomico ($t = 1.49$), are not significant at the five percent level.

When the different river systems are compared for both year classes (tables 19 and 20) it is obvious that the values for the James River are lower than for all others. Tests show that the samples very probably were not drawn from the same population. When the James sample for 1955 (table 19) is tested against the composite sample from the Elk to York, $X^2 = 51.12$ which is significant at the one percent level and indicates the two samples very probably were not drawn

from the same population. In a similar test for the 1954 sample (table 20) comparing the James sample with the composite sample from the Choptank to the York, $X^2 = 39.72$ which is significant at the one percent level and indicates probable heterogeneity.

When arranged in composite samples representing the East Shore, West Shore (James River excluded), and the James River in table 11, it is obvious that the latter sample differs and a test indicates that they very probably were not drawn from the same population. The West Shore sample differs slightly from that of the East Shore but there seems to be no biological significance.

The Chesapeake Bay samples are arranged in four geographic groups from north to south in table 12. The James sample is obviously different from each of the other three composite samples and a test indicates that the four samples very probably were not drawn from the same population.

In summary the results from an analysis of anal ray counts of Chesapeake Bay samples indicates the presence of two subpopulations.

A comparison of the anal soft rays of samples from the Hudson and the James Rivers is given in table 12. The two samples are significantly different at the one percent level and very probably were not drawn from the same population. An examination of the data in tables 13, 19 and 20, show that the Hudson sample is differentiated from other Chesapeake populations at a higher level than it is from the James sample.

DISCUSSION

Throughout the range of the striped bass the number of spines in the first dorsal fin is remarkably constant except for the high values for the James River samples. A study of the constancy of this character in several year classes through a considerable mileage of the James and Hudson Rivers shows it to be quite stable which would hardly be the case if it were influenced to any considerable extent by environmental fluctuations. This character alone indicates the presence of two well-defined subpopulations in the Chesapeake Bay area. For two year classes the James River, the most southerly drainage in Chesapeake Bay, has the high values which contrast with others but particularly with the nearby York-Rappahannock-Pocomoke systems. The latter three have in turn somewhat lower values than samples from the Upper Bay. The James sample is also much higher than the next most southerly sample from Albemarle Sound which confirms the returns from tagging experiments that show little exchange between the two populations. The James River subpopulation also differs in this regard from others studied along the Atlantic and Gulf coasts. It approaches but differs significantly from the Hudson population.

In contrast to the high value for first dorsal spines, the James River sample has low or moderate values for soft dorsal rays and soft anal rays. Because of this relationship between the fin spines and the soft ray counts seen above, a character or meristic index was determined for each specimen by subtracting for each specimen the number of first dorsal fin spines from the total number of soft dorsal and anal rays. The results for the 1955 year class in table 21 show the James sample to be markedly different from the York-Rappahannock samples and also to differ from the Upper Bay samples which have intermediate values. Chi-square gives a value of $X^2 = 128.14$ when the James sample is tested against the York-Rappahannock composite sample; $X^2 = 92.52$ when the James is tested against the Elk-Wicomico composite; and the latter when tested against the York-Rappahannock gives a value $X^2 = 21.74$. These values are significant at the one percent level, which indicates the samples very probably were not drawn from the same population. The data for the 1954 year class (table 22) also shows the James sample to be different from the other samples from the Bay. When it is tested against the composite Choptank-York sample, $X^2 = 114.88$ which is highly significant at the one percent level and indicates they very probably were not drawn from the same population.

It may be seen from the data given in table 21 that a line drawn between 13 and 14 of the character index permits a separation of 67 percent of the James sample (low value) from 95 percent of the York sample (high value) or an

average separation of 81 percent. A similar comparison of the James with the Rappahannock, which has a slightly lower mean value, separates 67 percent of the James specimens from 87 percent of those from the Rappahannock, for an average of 77 percent. When the James sample is compared with that of the Nanticoke, which has the lowest mean value except for the James, 67 percent of those from the James are separated from 68 percent of those from the Nanticoke, or an average of 67.5 percent.

For the 1954 year class a line drawn between character index numbers 13 and 14 (table 22) permits a separation of 66 percent of the James sample from 82 percent of the York sample, an average of 74 percent. Where the numbers are adequate for this type of treatment for the Upper Bay samples, the lowest mean value is found in the Wicomico, 66 percent of the James specimens may be separated from 64 percent of the Wicomico sample, an average of 65 percent.

Each of the fin ray characters studied furnish a statistical basis for the separation of the James River subpopulation from that in the adjacent York and Rappahannock Rivers. The latter subpopulation is also separable, on the basis of second dorsal soft rays, from the subpopulation of the Upper Bay tributaries except that of the Pocomoke. A meristic index based on first dorsal fin spines and on dorsal and anal soft rays supports this conclusion.

What is the cause of these differences; are they wholly or in part environmentally controlled? Evidence is provided that first dorsal spines are relatively constant throughout a wide range in the Hudson River and through a considerable geographic expanse of the James River. On this basis it is assumed that the character is perhaps less affected by environmental fluctuations than are some of the other fin ray counts.

Soft dorsal and anal rays show a trend toward an increase in downstream samples in the James River but the differences are usually not significant. A significant increase is noted in downstream samples in the Hudson River which perhaps are a reflection of average temperature and/or salinity differences. However, these character differences are relatively small compared with those which are considered to denote races. A more precise solution of the problem of the time of fixation of, and the effect of temperature and other factors on meristic characters in our important fishes awaits needed experiments of the type described by Tåning (1953).

How do the more direct results obtained by tagging contribute to the concept of three subpopulations in the tributaries of Chesapeake Bay? Pearson (1938: 842) reported on the returns of striped bass from 26 to 40 cm. long tagged in July and August, 1931 off Annapolis, Maryland. Over a two-year period 29.1 percent were recovered. Of 89 recoveries only 9 were taken south of the point where they were originally marked and released. Most were captured in the Upper Bay from Magothy River and Love Point north to the Susquehanna and Elk Rivers. The point of greatest concentration was in the vicinity of Rock Hall near the entrance to the Chester River. The most distant points of recovery were single specimens from the Wicomico and Potomac Rivers. Much additional data on migration within the Bay from the studies of Vladykov and Wallace (1938 and 1952) showed that striped bass tagged in the middle Bay area remained in the area where they were originally tagged during late summer and fall. However, it was a heterogeneous population and by the end of October some started to move southward, especially along the western shore of the Bay; a few reached the Rappahannock and James Rivers and some left the Bay. They concluded that the Choptank and Susquehanna Rivers were the main spawning areas for the bass originally tagged at Galesville, Flag Pond and Tilghman in the middle region of the Bay. Data for fin ray counts also support the view of an Upper Bay subpopulation. Of considerable numbers tagged in October in the Potomac River only a small percentage was recaptured elsewhere which may indicate a local population. The characters used in this study do not confirm this view.

Those tagged in the James River were almost all recaptured there and Vladykov and Wallace (1938 and 1952) assumed that this was a separate population. This finding is supported by the present study.

Previous tagging returns indicates little

interchange between the James River and Albemarle Sound. Meristic data, especially first dorsal fin spine counts, support this finding. A large tagging program by the U. S. Fish and Wildlife Service now underway on Albemarle Sound and its tributaries may provide data to further clarify this facet.

The detailed tagging results reported by Raney, Woolcott, and Mehring (1954) which gave further evidence of the racial separateness of the Hudson and Chesapeake populations are supported by the differences in the meristic characters reported herein (table 13).

SUMMARY AND CONCLUSIONS

1. It is concluded on the basis of meristic studies which are supported by findings of earlier tagging experiments that three subpopulations are present within the tributaries of Chesapeake Bay. The James subpopulation is best defined. The other two are the York-Rappahannock subpopulation and the Upper Bay subpopulation.

2. The number of first dorsal spines is fairly constant in samples from two year classes taken throughout a wide geographic range in both the James and Hudson Rivers and this appears to indicate little effect by environmental fluctuations.

3. The James subpopulation has high values for the dorsal spines and is statistically different from other populations in Chesapeake Bay and elsewhere along the Atlantic Coast including the population in Albemarle Sound, North Carolina.

4. Soft dorsal and anal rays show a slight but significant increase in downstream samples in the Hudson River. This may be a reflection of temperature and/or salinity differences.

5. In the James River downstream increases in dorsal and anal rays are not statistically significant.

6. The James subpopulation is significantly different from the adjacent York-Rappahannock subpopulation and from the Upper Bay subpopulation in having lower values for soft dorsal rays and soft anal rays.

7. The York-Rappahannock subpopulation differs from the Upper Bay subpopulation in second dorsal soft rays (table 12) but these subpopulations are not as highly differentiated as are those of the James and York-Rappahannock.

8. Both the Hudson and James populations approach and differ from other Chesapeake Bay tributaries in having a high dorsal spine count and low dorsal soft and low anal soft ray counts (table 12), but they are significantly different. This supports the former meristic and tagging studies which regarded the Hudson and Chesapeake populations as separate races.

9. Where several characters are correlated the meristic or character index continues to be a useful technique in separating populations (tables 21-22).

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Figure 1.--Chesapeake Bay and its main tributaries.

Table 1.--Variation with size (standard length) in the number of dorsal spines and soft rays, and anal soft rays: young striped bass taken (217 specimens taken on July 23 and 24) in the James River, Virginia.

No. of spines in first dorsal	No. of specimens	Standard length (mm.) Range	Mean	S D.	S.E.
9 or fewer	150	35-89	51.67	9.67	0.79
10 or more	69	39-71	50.94	6.87	0.83
t = 0.56; not significant at the 5 percent level					
No. of dorsal soft rays					
11 or fewer	96	38-82	51.50	8.77	0.90
12 or more	123	35-89	51.44	9.06	0.82
t = 0.05; not significant at the 5 percent level					
No. of anal soft rays					
10 or fewer	58	38-80	50.02	7.12	0.93
11 or more	161	35-89	51.99	9.39	0.74
t = 1.45; not significant at the 5 percent level					

Table 2.--Frequency of number of spines and soft rays in the dorsal and anal fins of young striped bass (taken on July 21 and 22, 1955) in the James River, Virginia.

Locality	No. of specimens	First dorsal spines 8	9	10	11	Mean	$S(x-\bar{x})^2$
Windmill Pt., J55 [1/]	59	--	45	11	3	9.29	18.102
Bachelors Pt., J47.5	100	1	74	24	1	9.25	22.750
Sloop Pt., J43	34	--	22	10	2	9.41	12.235
Chickahominy R. mouth, C43	27	--	20	6	1	9.30	7.630
Swan Pt., J35	58	1	44	13	-	9.21	11.517
Hog Is. J29	39	1	28	8	2	9.28	13.898
X^2 = 2.12, N.S.							
	No. of specimens	Second dorsal soft rays 10	11	12	13	Mean	$S(x-\bar{x})^2$
Windmill Pt.	60	1	32	26	1	11.45	18.850
Bachelors Pt.	100	2	48	49	1	11.49	30.990
Sloop Pt.	34	--	14	20	--	11.59	8.235
Chickahominy R. mouth	27	--	12	15	--	11.56	6.667
Swan Pt.	58	1	23	34	--	11.57	16.224
Hog Is.	39	2	14	23	--	11.54	13.692
X^2 = 3.73, N.S.							

Table 2 continued on next page

Locality	No. of specimens	Anal soft rays 9	10	11	12	Mean	$S(x-\bar{x})^2$
Windmill Pt.	60	1	14	44	1	10.75	15.250
Bachelors Pt.	100	--	23	77	--	10.77	17.710
Sloop Pt.	34	--	6	28	--	10.82	4.941
Chickahominy R. mouth	27	--	3	24	--	10.89	2.667
Swan Pt.	57	--	19	38	--	10.67	12.667
Hog Is.	39	1	12	25	1	10.67	12.667
X^2 = 7.54, N.S.							

1/ Distance in nautical miles from the mouth of the James River

Table 3.--Frequency of number of spines and soft rays in the dorsal and anal fins of composite upstream and downstream samples of young striped bass in the James River, Virginia.

1/Taken on July 21 and 22, 1955. The upstream sample includes Windmill Pt. (J55) and Bachelors Pt. (J47.5); the downstream sample Sloop Pt. (J43), Chickahominy R. mouth (C43), Swan Pt. (J35) and Hog Is. (J29).1/

Sample	No. of specimens	First dorsal spines 8	9	10	11	Mean	$S(x-\bar{x})^2$
Upstream	159	1	119	35	4	9.26	40.906
Downstream	158	2	114	37	5	9.28	46.184
X^2 = 0.18, N.S.							

	No. of specimens	Second dorsal soft rays 10	11	12	13	Mean	$S(x-\bar{x})^2$
Upstream	160	3	80	75	2	11.48	49.900
Downstream	158	3	63	92	--	11.56	44.867
X^2 = 2.86, N.S.							

	No. of specimens	Anal soft rays 9	10	11	12	Mean	$S(x-\bar{x})^2$
Upstream	160	1	37	121	1	10.76	32.975
Downstream	157	1	40	115	1	10.74	34.298
X^2 = 0.38, N.S.							

Table 4.--Frequency of number of spines and soft rays in the dorsal and anal fins in composite upstream and downstream samples of young striped bass in the James River, Virginia.

/Taken on July 23 and 24, 1954. The upstream sample includes Berkley (J60), Westover (J59), Coggins Pt. (J59), Wilcox Wharf (J56) and Bachelors Pt. (J47.5). Downstream sample: Dancing Pt. (J42), Chickahominy R. mouth (C43), Barrets Pt. (J41), Back River (J35), and Treasure Island Beach (J30)/

		First dorsal spines					
Sample	No. of specimens	8	9	10	11	Mean	$S(x-\bar{x})^2$
Upstream	122	1	92	22	7	9.29	40.959
Downstream	56	--	39	16	1	9.32	14.214
X^2 = 0.55, N.S.							

		Second dorsal soft rays						
	No. of specimens	9	10	11	12	13	Mean	$S(x-\bar{x})^2$
Upstream	120	1	6	53	59	1	11.44	49.592
Downstream	55	--	--	18	37	--	11.67	12.109
X^2 = 3.88, significant at the 5 percent level								

		Anal soft rays			
	No. of specimens	10	11	Mean	$S(x-\bar{x})^2$
Upstream	121	36	85	10.70	25.289
Downstream	44	10	34	10.77	7.727
X^2 = 0.48, N.S.					

Table 5.--Number of spines and soft rays in the dorsal and anal fins of young striped bass in the Chickahominy River, Virginia.

/Taken on July 23, 1954/

		First dorsal spines				
Locality	No. of specimens	9	10	11	Mean	$S(x-\bar{x})^2$
Shackleford Farm, C55	41	18	20	3	9.63	15.512
Above bridge near mouth, C43	20	16	4	--	9.20	3.200
X^2 = 8.63, significant at the 1 percent level						

		Second dorsal soft rays				
	No. of specimens	10	11	12	Mean	$S(x-\bar{x})^2$
Shackleford Farm	41	1	14	26	11.61	11.756
Above bridge near mouth	20	--	6	14	11.70	4.200
X^2 = 0.05						

		Anal soft rays					
	No. of specimens	8	9	10	11	Mean	$S(x-\bar{x})^2$
Shackleford Farm	41	1	2	8	30	10.63	19.512
Above bridge near mouth	20	--	--	3	17	10.85	2.550
X^2 = 0.50, N.S.							

Table 6.--Frequency of number of spines in the first dorsal fin of young striped bass of the 1954 year class taken in the Hudson River, New York

Locality	No. of specimens	First dorsal spines 7	8	9	10	11	12	Mean	$S(x-\bar{x})^2$
Coxsackie	125	--	2	113	10	--	--	9.06	11.488
Middleground Is.	20	--	--	20	--	--	--	9.00	0.000
Alsen	56	--	--	48	8	--	--	9.14	6.857
Staatsburg	76	--	--	62	14	--	--	9.18	11.421
Newburgh	93	1	1	81	8	1	1	9.11	24.925
Beacon	95	--	1	80	14	--	--	9.14	13.221
Peekskill	100	--	1	90	9	--	--	9.08	9.360
Grassy Pt.	96	--	1	84	11	--	--	9.10	10.958
Haverstraw	234	--	--	211	23	--	--	9.10	20.739
Harmon	212	--	1	193	18	--	--	9.08	17.637
Croton Pt.	93	--	--	79	12	2	--	9.17	17.247
Nyack	132	--	1	116	13	2	--	9.12	20.061
Piermont	52	--	--	47	5	--	--	9.10	4.519
Palisades	96	--	--	84	11	1	--	9.14	13.240

X^2 = 13.85, N.S.

Table 7.--Frequency of number of spines and soft rays in the dorsal and anal fins of young striped bass of the 1954 year class taken in the Hudson River, New York.

/The upstream sample includes those from Coxsackie to Beacon and the downstream sample those from Peekskill to Palisades./

Sample	No. of specimens	First dorsal spines 7	8	9	10	11	12	Mean	$S(x-\bar{x})^2$
Upstream	465	1	4	404	54	1	1	9.11	68.959
Downstream	1015	-	4	904	102	5	--	9.11	114.508

X^2 = 0.89, N.S.

	No. of specimens	Second dorsal soft rays 8	9	10	11	12	13	14	Mean	$S(x-\bar{x})^2$
Upstream	465	1	4	38	313	106	2	1	11.14	177.19
Downstream	1021	-	1	45	605	354	15	1	11.33	358.78

X^2 = 23.37, significant at the 1 percent level

	No. of specimens	Anal soft rays 7	8	9	10	11	12	Mean	$S(x-\bar{x})^2$
Upstream	464	2	2	7	211	241	1	10.49	167.922
Downstream	1022	1	3	12	363	639	4	10.61	304.560

X^2 = 14.90,

Table 8.--Frequency of number of spines and soft rays in the dorsal and anal fins of young striped bass of the 1953 year class taken in August and September, 1953 in the Hudson River, New York

Locality	No. of specimens	First dorsal spines 8	9	10	Mean	$S(x-\bar{x})^2$
Coxsackie	64	-	59	5	9.08	4.609
Haverstraw	84	1	76	7	9.07	7.572

X^2 = 0.04, N.S.

	No. of specimens	Second dorsal soft rays 10	11	12	13	Mean	$S(x-\bar{x})^2$
Coxsackie	64	2	47	15	--	11.20	14.359
Haverstraw	70	--	34	35	1	11.53	19.443

X^2 = 9.96, significant at the 1 percent level

	No. of specimens	Anal soft rays 9	10	11	Mean	$S(x-\bar{x})^2$
Coxsackie	64	1	27	36	10.55	17.859
Haverstraw	70	--	15	55	10.79	11.786

X^2 = 6.65, significant at the 1 percent level

Table 9. -Frequency of number of spines in the first dorsal fin of young striped bass of the 1955 year class from the tributaries of Chesapeake Bay. /East shore tributaries are indicated by an asterisk./

River		No. of specimens	First dorsal spines 8	9	10	11	12	Mean	$S(x-\bar{x})^2$
Elk*	A	49	--	44	4	--	1	9.14	12.000
Chester*	B	37	--	35	2	--	--	9.05	1.892
Choptank*	C	75	--	64	10	1	--	9.16	12.080
Nanticoke*	D	26	--	23	2	1	--	9.15	5.385
Wicomico*	E	70	--	67	3	--	--	9.04	2.872
Rappahannock	F	118	--	113	5	--	--	9.04	4.788
York	G	65	1	63	1	--	--	9.00	2.000
James	H	320	3	235	73	9	--	9.28	87.800

X^2 = 59.22, significant at the 1 percent level

Table 10.--Frequency of number of spines in the first dorsal fin of young striped bass of the 1954 year class from the tributaries of Chesapeake Bay

River		No. of specimens	First dorsal spines 8	9	10	11	12	Mean	$S(x-\bar{x})^2$
Choptank*	A	41	--	40	1	--	--	9.02	0.976
Patuxent	B	34	--	32	2	--	--	9.06	1.882
Fishing Bay*	C	11	--	10	1	--	--	9.09	0.909
Nanticoke*	D	178	1	166	10	1	--	9.06	14.320
Wicomico*	E	59	--	53	6	--	--	9.10	5.390
Potomac	F	95	--	84	10	1	--	9.13	12.484
Pocomoke*	G	21	--	21	--	--	--	9.00	0.000
Rappahannock	H	63	2	57	4	--	--	9.03	5.937
York	I	233	--	230	3	--	--	9.01	2.961
James	J	219	1	149	58	11	--	9.36	74.502

X^2 = 125.53, significant at the 1 percent level.

Table 11.--Frequency of number of spines and soft rays in the dorsal and anal fins in composite samples of young striped bass of the 1954 and 1955 year classes from Chesapeake Bay tributaries arranged geographically

Locality		No. of specimens	First dorsal spines 8	9	10	11	12	Mean	$S(x-\bar{x})^2$
E. Shore tributaries	A	578	1	533	40	3	1	9.08	58.014
W. Shore tributaries (except James R.)	B	614	3	585	25	1	--	9.04	31.062
James R.	C	539	4	384	131	20	--	9.31	163.258

X^2 = 166.45, significant at the 1 percent level

		No. of specimens	Second dorsal soft rays 9	10	11	12	13	14	Mean	$S(x-\bar{x})^2$
E. Shore tributaries	A	578	--	6	148	415	9	--	11.74	141.552
W. Shore tributaries (except James R.)	B	612	--	4	74	519	14	1	11.89	100.882
James R.	C	537	1	13	228	292	3	--	11.53	171.858

X^2 = 150.32, significant at the 1 percent level

		No. of specimens	Anal soft rays 8	9	10	11	12	Mean	$S(x-\bar{x})^2$
E. Shore tributaries	A	578	-	2	61	509	6	10.90	68.978
W. Shore tributaries (except James R.)	B	614	-	1	36	565	12	10.96	50.899
James R.	C	442	1	4	106	329	2	10.74	103.079

X^2 = 86.48, significant at the 1 percent level

Table 12.--Frequency of number of spines and soft rays in the dorsal and anal fins in composite samples of young striped bass of the 1954 and 1955 year classes from Chesapeake Bay tributaries arranged geographically from north to south

Locality		No. of specimens	First dorsal spines 8	9	10	11	12	Mean	$S(x-\bar{x})^2$
Elk to Chester	A	100	--	92	7	--	1	9.10	15.000
Choptank to Potomac and Pocomoke	B	613	1	563	45	4	--	9.08	57.589
Rappahannock and York	C	479	3	463	13	--	--	9.02	15.791
James	D	539	4	384	131	20	--	9.31	163.258

X^2 = 170.57, significant at the 1 percent level

		No. of specimens	Second dorsal soft rays 9	10	11	12	13	14	Mean	$S(x-\bar{x})^2$
Elk to Chester	A	94	--	2	20	72	--	--	11.74	21.872
Choptank to Potomac and Pocomoke	B	607	--	8	155	431	12	1	11.74	162.392
Rappahannock and York	C	480	--	--	44	426	10	--	11.93	51.592
James	D	537	1	13	228	292	3	--	11.53	171.858

X^2 = 165.25, significant at the 1 percent level

		No. of specimens	Anal soft rays 8	9	10	11	12	Mean	$S(x-\bar{x})^2$
Elk to Chester	A	100	--	--	9	91	--	10.91	8.190
Choptank to Potomac and Pocomoke	B	613	--	2	63	540	8	10.90	73.321
Rappahannock and York	C	479	--	1	25	443	10	10.96	38.397
James	D	442	1	4	106	329	2	10.74	103.079

X^2 = 86.63, significant at 1 percent level

Table 13.--Frequency of number of spines and soft rays in the dorsal and anal fins of young striped bass of the 1954 year class from the Hudson and James Rivers

River	No. of specimens	First dorsal spines 7	8	9	10	11	12	Mean	$S(x-\bar{x})^2$
Hudson	1480	1	8	1308	156	6	1	9.11	183.486
James	219	--	1	149	58	11	--	9.36	74.502

X^2 = 66.22, significant at the 1 percent level

	No. of specimens	Second dorsal soft rays 8	9	10	11	12	13	14	Mean	$S(x-\bar{x})^2$
Hudson	1486	1	5	83	918	460	17	2	11.27	548.164
James	216	--	1	7	85	122	1	--	11.53	75.773

X^2 = 49.29, significant at the 1 percent level

	No. of specimens	Anal soft rays 7	8	9	10	11	12	Mean	$S(x-\bar{x})^2$
Hudson	1486	3	5	19	574	880	5	10.57	469.145
James	216	--	1	2	54	159	--	10.72	53.773

X^2 = 15.12, significant at the 1 percent level

Table 14.--Frequency of number of soft rays in the second dorsal and anal fins of young striped bass taken on July 23 and 24, 1954 in the James River, Virginia.

Locality	No. of specimens	Second dorsal soft rays 9	10	11	12	13	Mean	$S(x-\bar{x})^2$
Berkley, J60, Westover, J59 Coggins Pt., J59	27	--	1	14	12	--	11.41	8.519
Wilcox Wharf, J56	18	--	2	6	10	--	11.44	8.444
Bachelors Pt., J47.5	75	1	3	33	37	1	11.45	32.587
Dancing Pt., J42, Barrets Pt., J41	11	--	--	4	7	--	11.64	2.546
Back River, J35	14	--	--	4	10	--	11.71	2.857
Treasure Is. Beach, J30	10	--	--	4	6	--	11.60	2.400
Chickahominy R. Shackleford Farm, C55	41	--	1	14	26	--	11.61	11.756
Above bridge near mouth, C43	20	--	--	6	14	--	11.70	4.200

$X^2 = 6.47$, N.S.

	No. of specimens	Anal soft rays 8	9	10	11	Mean	$S(x-\bar{x})^2$
Berkley, Westover, Coggins Pt.	28	--	--	14	14	10.50	7.000
Wilcox Wharf	18	--	--	6	12	10.67	4.000
Bachelors Pt.	75	--	--	16	59	10.79	12.587
Dancing Pt., Barrets Pt.	11	--	--	4	7	10.64	2.546
Back R.	13	--	--	2	11	10.85	1.692
Treasure Is. Beach	10	--	--	1	9	10.90	0.900
Chickahominy R. Shackleford Farm	41	1	2	8	30	10.63	19.512
Above bridge near mouth	20	--	--	3	17	10.85	2.550

$X^2 = 13.57$, N.S.

Table 15.--Frequency of number of soft rays in the second dorsal fin of young striped bass of the 1954 year class taken in the Hudson River, New York

Locality	No. of specimens	Second dorsal soft rays 8	9	10	11	12	13	14	Mean	$S(x-\bar{x})^2$
Coxsackie	125	--	4	9	78	33	--	1	11.15	64.112
Middleground Is.	20	--	--	1	14	5	--	--	11.20	5.200
Alsen	56	1	--	6	35	13	1	--	11.11	31.357
Staatsburg	76	--	--	7	51	18	--	--	11.14	23.408
Newburgh	93	--	--	6	70	16	1	--	11 13	24.452
Beacon	95	--	--	9	65	21	--	--	11.13	28.484
Peekskill	100	--	--	6	57	35	2	--	11.33	38.110
Grassy Pt.	96	--	--	2	69	25	--	--	11.24	21.490
Haverstraw	236	--	--	12	138	80	6	--	11.34	88.881
Harmon	213	--	--	16	130	66	1	--	11.24	73.305
Croton Pt.	93	--	--	1	54	35	3	--	11.43	30.796
Nyack	134	--	--	4	77	49	3	1	11.40	52.239
Piermont	53	--	--	3	25	25	--	--	11.42	18.868
Palisades	96	--	1	1	55	39	--	--	11.38	30.500

$X^2 = 38.22$, significant at the 1 percent level

Table 16.--Frequency of number of soft rays in the second dorsal fin of young striped bass of the 1955 year class from tributaries of Chesapeake Bay

River		No. of specimens	Second dorsal soft rays 10	11	12	13	Mean	$S(x-\bar{x})^2$
Elk*	A	49	1	13	35	--	11.69	12.408
Chester*	B	37	1	5	31	--	11.81	7.676
Choptank*	C	75	--	24	50	1	11.69	17.947
Nanticoke*	D	28	2	6	20	--	11.65	10.429
Wicomico*	E	70	--	25	43	2	11.67	19.443
Rappahannock	F	118	--	8	109	1	11.94	8.585
York	G	65	--	--	64	1	12.02	0.985
James	H	321	6	143	170	2	11.52	96.075

X^2 = 102.56, significant at the 1 percent level

Table 17.--Frequency of number of soft rays in the second dorsal fin of young striped bass of the 1954 year class from tributaries of Chesapeake Bay

River	No. of specimens	Second dorsal soft rays 9	10	11	12	13	14	Mean	$S(x-\bar{x})^2$
Choptank*	41	--	--	4	36	1	--	11.93	4.780
Patuxent	34	--	1	6	26	1	--	11.79	9.559
Fishing Bay*	11	--	--	3	8	--	--	11.73	2.182
Nanticoke*	176	--	2	44	128	2	--	11.74	41.977
Wicomico*	59	--	--	17	40	2	--	11.75	15.186
Potomac	92	--	3	23	62	3	1	11.74	35.739
Pocomoke*	21	--	--	3	18	--	--	11.86	2.572
Rappahannock	63	--	--	12	48	3	--	11.86	13.714
York	234	--	--	24	205	5	--	11.92	27.457
James	216	1	7	85	122	1	--	11.53	75.773

X^2 = 74.07, significant at the 1 percent level

Table 18.--Frequency of number of soft rays in the anal fin of young striped bass of the 1954 year class taken in the Hudson River, New York

Locality	No. of specimens	Anal soft rays 7	8	9	10	11	12	Mean	$S(x-\bar{x})^2$
Coxsackie	125	1	2	2	47	73	--	10.51	59.232
Middleground Is.	20	--	--	1	9	10	--	10.45	6.950
Alsen	55	1	--	1	25	28	--	10.44	27.527
Staatsburg	76	--	--	2	31	42	1	10.55	24.790
Newburgh	93	--	--	--	54	39	--	10.42	22.645
Beacon	95	--	--	1	45	49	--	10.50	25.747
Peekskill	99	--	2	1	34	60	2	10.60	41.838
Grassy Pt.	96	--	--	2	38	56	--	10.56	27.625
Haverstraw	236	--	1	3	79	152	1	10.63	68.928
Harmon	215	1	--	2	85	126	1	10.57	70.633
Croton Pt.	93	--	--	--	27	66	--	10.71	19.161
Nyack	134	--	--	1	53	80	--	10.59	34.425
Piermont	53	--	--	1	21	31	--	10.57	15.019
Palisades	96	--	--	2	26	68	--	10.69	24.625

X^2 = 30.63, significant at the 1 percent level

Table 19.--Frequency of number of soft rays in the anal fin of young striped bass from the 1955 year class from tributaries of Chesapeake Bay

River		No. of specimens	Anal soft rays 9	10	11	12	Mean	$S(x-\bar{x})^2$
Elk*	A	49	--	5	44	--	10.90	4.490
Chester*	B	37	--	2	35	--	10.95	1.892
Choptank*	C	75	--	7	68	--	10.91	6.347
Nanticoke*	D	28	--	1	27	--	10.96	0.964
Wicomico*	E	68	1	4	62	1	10.93	8.632
Rappahannock	F	118	--	3	113	2	10.99	4.992
York	G	65	--	2	62	1	10.98	2.985
James	H	226	2	52	170	2	10.76	49.097

X^2 = 51.12, significant at the 1 percent level

Table 20.--Frequency of number of soft rays in the anal fin of young striped bass from the 1954 year class from tributaries of Chesapeake Bay

River	No. of specimens	Anal soft rays 8	9	10	11	12	Mean	$S(x-\bar{x})^2$
Choptank*	41	--	--	3	38	--	10.93	2.780
Patuxent	34	--	--	2	31	1	10.97	2.971
Fishing Bay*	11	--	--	1	10	--	10.91	0.909
Nanticoke*	178	--	1	23	149	5	10.89	29.753
Wicomico*	59	--	--	10	49	--	10.83	8.305
Potomac	95	--	--	9	85	1	10.92	9.326
Pocomoke*	21	--	--	3	18	--	10.86	2.572
Rappahannock	63	--	--	4	59	--	10.94	3.746
York	233	--	1	16	209	7	10.95	26.481
James	216	1	2	54	159	--	10.72	53.773

X^2 = 45.80, significant at the 1 percent level

Table 21.--Frequency distributions of the character index (first dorsal spines subtracted from the sum of dorsal and anal soft rays) for samples of young striped bass of the 1955 year class from the tributaries of Chesapeake Bay

River		No. of specimens	Character index 10	11	12	13	14	15	Mean	$S(x-\bar{x})^2$
Elk*	A	49	1	--	4	14	30	--	13.47	32.204
Chester*	B	37	--	--	2	7	28	--	13.70	11.730
Choptank*	C	75	--	--	8	29	37	1	13.41	36.187
Nanticoke*	D	28	--	1	5	4	18	--	13.39	22.679
Wicomico*	E	70	--	2	2	24	40	2	13.54	37.372
Rappahannock	F	93	--	--	1	11	79	2	13.88	15.699
York	G	65	--	--	--	3	59	3	14.00	6.000
James	H	230	--	16	47	91	72	4	13.00	198.996

X^2 = 135.56, significant at the 1 percent level

Table 22.--Frequency distributions of the character index (first dorsal spines subtracted from the sum of dorsal and anal soft rays) for samples of young striped bass of the 1954 year class from tributaries of Chesapeake Bay

River		No. of specimens	Character index 9	10	11	12	13	14	15	16	Mean	$S(x-\bar{x})^2$
Choptank*	A	87	--	--	--	1	9	76	1	--	13.88	12.851
Patuxent	B	35	--	--	--	2	8	23	2	--	13.71	15.143
Fishing Bay*	C	19	--	--	--	2	6	11	--	--	13.47	8.737
Nanticoke*	D	213	--	1	6	16	44	139	7	--	13.57	146.122
Wicomico*	E	59	--	--	1	9	11	37	1	--	13.47	40.712
Potomac	F	37	--	--	2	3	9	22	1	--	13.46	29.189
Pocomoke*	G	21	--	--	--	1	4	16	--	--	13.71	6.286
Rappahannock	H	91	--	--	1	3	18	65	4	--	13.75	37.187
York	I	129	1	--	2	1	19	97	9	--	13.82	70.899
James	J	218	1	10	13	45	74	74	1	--	12.87	277.142

X^2 = 134.72, significant at the 1 percent level

THE SUBPOPULATION PROBLEM IN THE PACIFIC SARDINE SARDINOPS CAERULEA

By

John C. Marr[1/]

The fishery for the Pacific sardine (Sardinops caerulea) is one of the most completely documented in the world and, similarly, knowledge of the biology of the sardine is at least as complete as it is for any other marine fish. Information arising from a long period of study, plus greatly intensified studies in recent years, now makes it possible to ask intelligent questions about the number and location of sardine subpopulations and to suggest critical methods of examining these questions. The information now at hand, the questions asked and methods of seeking answers to them will be reviewed in the following sections.

The introductory section of the first paper in this collection is pertinent here and need not be repeated. Suffice it to say that I use the term "subpopulation" in the sense that it is a self-sustaining unit; subpopulations segregate at spawning time and their characteristics are heritable.

REVIEW OF PRESENT KNOWLEDGE

Many kinds of information bearing directly or indirectly on the subpopulation problem have accumulated over the years. These are categorized below.

Catch data and other information: Twenty years ago it was generally believed that there was only one major group of sardines, which was produced in the southern part of its range and, with increasing size (or age), performed successively longer feeding migrations to the north in the spring and summer and spawning migrations to the south in the fall and winter. (The sardines off the west coast of southern Lower California and in the Gulf of California were considered to be of uncertain relationship to the northern group.) This view was summarized by Clark (1935:5-6):

"The changes in size of fish from the smaller, fall fish to the larger, winter fish result from the movements of sardines up and down the California coast. Young fish reared on the nursery grounds of southern California and northern Lower California work northward during their first and second years. The spring bait fishery at San Diego appears to be composed in part of fish which have been reared at San Diego and in part of fish which have been reared to the southward. At approximately two years of age (7-1/2 to 9-1/2 inches in length) sardines of any year class are first taken in important numbers in the San Pedro fall fishery. Some fish of this size and age also appear at Monterey in the fall of the same year but the majority does not go as far north as Monterey until one year later. These fish are adolescent and in general exhibit the same behavior as do the adult sardines. During the summer of each succeeding year a given year class moves a little farther north along the California coast and makes a return journey to the south during the fall and winter. The more extended northward movement each succeeding summer causes a correspondingly later appearance of the older fish at any given point on the southward journey. The larger and older fish do not appear off Monterey and San Pedro until the winter months. Their return to Monterey occurs about a month earlier than to San Pedro. Just how far north any year class moves in each succeeding summer we have not learned as yet, but all evidence at hand indicates that the largest and oldest fish reach British Columbia sometime between July and September. Perhaps these fish constitute stragglers from the main population but more probably the majority of them again returns to southern California waters in the spring to

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deposit their eggs on the main spawning grounds south of Point Conception. This north and south movement seemingly is a feeding and spawning migration: northward during the spring and summer, when the sardine is feeding and storing up fat, and southward during the fall and winter with the sardine still feeding and the sex products maturing preparatory to another spawning in southern waters."

In support of this general theory Clark (1935:6) states:

"The evidence to substantiate these conclusions about the sardine movements is: the apparent homogeneity of the population along the entire coast (Hubbs, 1925; Clark, F.N., 1931; Hart, 1933.2) the greater abundance of smaller, younger fish in the south than in the north as shown by the bait and quarter-oil fisheries and the San Pedro and Monterey cannery catches; the occurrence of the largest, oldest fish during the summer in British Columbia, their appearance first at San Francisco in the early winter, then at Monterey and last at San Pedro and San Diego in the late winter (Higgins, 1926; Scofield, W.L., 1926.2; Clark, F.N., 1930.2; Hart, 1933.1); the maturation of the sex products at approximately the same rate as the southward movement followed by the first appearance of spent fish to the northward (Clark, F.N., 1934); and the location of the main spawning grounds south of Point Conception (Scofield, E.C., 1934)."

However, more recent work (to be discussed below) has shown that such a general explanation can, at the most, be only partially correct and that the true situation may be more complex.

Morphometric studies: Morphometric data were used by Thompson (1926) in comparing the local sardine with Atlantic (European) pilchard. He made no comparison between local samples.

Similar data were used by Taranets (1937) in comparing sardines from the eastern and western North Pacific.[2/]

No further use of morphometric data in attempts to distinguish subpopulations have been included in published reports, although there are references to such studies (Hart, 1934: H68, for example).

McHugh (1950--a doctoral dissertation, of which the parts relating to the sardine have not been published) gives data on the relative head length, predorsal length, preanal length

2/ According to Taranets (1937), whose work seems to have been rather generally overlooked, the two forms are con-specific and the form found off our coast should be known as Sardinops melanesticta caerulea (Girard). At the moment this seems to be a question to which there can only be a subjective answer, since there is no evidence to indicate that there is any genetic exchange between the two groups. (Of course, as Taranets points out, during previous periods of Arctic warming such gene flow obviously did take place, and presumably, in future periods of Arctic warming would again do so, unless the specific level of divergence has been attained prior to such warming.)

In a recent monograph of the Clupeidae, Svetovidov (1952) considers the eastern Pacific form to be Sardinops sagax caerulea (Girard). Earlier, Whittaker (1932) rejected Hubbs' (1929) erection of Sardinops and concluded, on the basis of anatomical studies, that the differences between Sardina and Sardinops were not of generic rank.

The taxonomic problem of whether or not these two genera are distinct, and the associated nomenclatorial problem, must be decided subjectively and has no bearing on this discussion. Similarly, the taxonomic problem of whether the sagax-neopilchardus-ocellata-caerulea-melanosticta complex represents an allopatric group of species or a group of incipient species and the associated nomenclatorial problem, must, on the basis of present knowledge, be decided subjectively. These questions are, at the moment, not pertinent to the population dynamics of the form inhabiting the northeastern Pacific. Without attempting to answer these taxonomic and nomenclatorial problems and in conformance with general usage in North America, I refer to the local form as Sardinops caerulea.

and prepelvic length of larval and juvenile sardine. In comparing relatively small samples from southern California and Lower California he found: (1) the head of larvae and juveniles from Lower California tends to be longer, deeper and have relatively bigger eyes than those from southern California. This tendency is eventually reversed, so that the adults from southern California have relatively longer heads than adults from Lower California. (2) The predorsal length is shorter in young fish from Lower California, but about the same in adults from the two localities. (3) There is only a slight tendency for fish from southern California to have a longer preanal distance. (4) The prepelvic length is slightly greater in sardines from Lower California. These findings are of interest, but more intensive work is needed to determine whether or not these differences may be characteristic of different subpopulations.

Dr. Wilhelm Harder, Institut für Fischereibiologie, Universität Hamburg, is engaged in a comparative study of the visceral morphology of clupeoids. While these particular findings have not yet been published, he has kindly informed me that there are certain differences in sardines from different localities. Most outstanding among these is the length of the small intestine in relation to standard length. At fish sizes of 100-150 mm. the small intestine is longer in sardines collected off British Columbia than in those collected off southern California and Lower California. At fish sizes of 175-225 mm. sardines collected off British Columbia have shorter small intestines than do fish from more southerly localities. In other words, although the small intestine of British Columbia sardines is longer at fish size 100 mm., its rate of increase relative to fish size is less than in fish from southern California and Lower California.

The cause of these differences and their physiological implications are as yet unknown.

A revision of the genus Sardinops is now in progress by R. W. Wisner, Scripps Institution of Oceanography. This study employs the conventional form of analysis of morphometric and meristic characters. It does not now seem likely that this revision will include the study of possible subpopulations of S. caerulea.

Meristic studies: (1) Vertebral numbers: Prior to the work of Schmidt (1917), average differences in meristic characters, such as vertebral numbers, were regarded as indicative of genetic differences between subpopulations of fishes. Following Schmidt's experiments and observations in nature by Hubbs (1934) and others, it was realized that while these characters undoubtedly have genetically determined limits they show great variability within these limits. This variability is presumably influenced by variations in environmental conditions, among which temperature has been most frequently mentioned.

Although this general problem would seem almost ideally susceptible of experimental analysis, very little progress has been made, owing in part at least, to the difficulty of rearing marine fishes. The recent experiments of Tåning (1944, 1946 and 1952) on sea trout, Salmo trutta trutta, will give impetus to such studies. His work indicates that the influence of temperature on vertebral number in sea trout is not a simple one and that other factors, such as oxygen pressure and carbon dioxide pressure, may also influence vertebral number.

Similar experiments on sardines are needed and will be carried out when methods of rearing marine fishes are available. It seems a reasonable a priori assumption that the variation in vertebral number exhibited by sardines will prove to be largely phenotypic. Evidence for this is offered by Clark (1947) and by McHugh (1950).

A number of studies, or continuing studies, have been made of variations in vertebral numbers of sardines. These include the work of Hubbs (1925), Thompson (1926), Hart (1933, 1934), Clark (1936, 1947), Taranets (1937), McHugh (1950), and California Marine Research Committee (1950). As early as 1934, Hart (1934:H67) concluded that "In view of the fact that the results are inconclusive in spite of the large amount of data collected, it would appear that other methods of studying this problem must be developed."

Clark (1947) has gathered and reported upon all available data (slightly more than 50,000 counts made on specimens from various localities from Alaska to the Gulf of California).

Her conclusions (1947:5-6) are:

"These vertebral counts indicate that sardines from British Columbia to Pt. San Eugenio in central Lower California comprise a mixture of populations, the young of which may have been reared on nursery grounds in any of these localities. For most seasons, presumably, the nursery grounds off California and northern Lower California make the greatest contributions to the population.

"Sardines living off southern Lower California and in the Gulf of California probably comprise a distinct group which does not mix with the northern fish; or if a mixture occurs, the proportion of southern fish to the total northern population is small.

"The interchange between nursery grounds begins early, perhaps before the sardines are a year old.

"The number of vertebrae varies between year-classes, and certain year-classes are characterized by high or low averages in all localities.

"The average number of vertebrae is approximately 51.7 for all sardines north of southern Lower California."

Although it was not specifically stated, Clark's use of the word "population" indicates an association of fish which have some distinct character, specifically, some unique average number of vertebrae. (This is what I have called a group.)

McHugh (1950) has presented additional data on vertebral numbers in sardines. Using collections of "postlarval" sardines from northern California, southern California, northern Lower California and southern Lower California, he demonstrated the latitudinal differences previously noted by others. He also found seasonal differences within a single geographical area which exceeded in magnitude the differences observed between areas

(2) Other meristic characters: Although meristic studies of sardines have been based largely on vertebral counts, some use has been made of other characters. Taranets (1937) compared the number of transverse rows of scales and the number of gill rakers in sardines from the eastern and western North Pacific. He did not consider differences within the local population.

The California Marine Research Committee (1950:42) briefly mentions: "Studies of other characters, such as the numbers of fin-rays and of gill-rakers, now under way, so far suggest that intermingling is not complete between Southern /sic/ California and the regions to the north." Presumably, this refers to work now being carried out by Wisner. As mentioned above, it now appears likely that this work will not be pertinent to studies at the subpopulation level.

McHugh (1950:67) has examined "unpublished data on dorsal and anal fin-ray counts of adult and young /sardines/ made by H.C. Godsil and W.E. Barraclough."[3/] According to McHugh's analysis of these data, the anal fin ray numbers show the presence of more than one group in the area from British Columbia to southern California and more than one group in the area south of southern California. The mean number of anal fin rays increased from British Columbia to southern California and decreased south of southern California.

The data on numbers of dorsal fin rays showed no consistent variation and McHugh suggests that counting errors may be responsible for this.

With regard to morphometric, meristic and perhaps other characteristics, it is pertinent to reiterate that these may be considerably modified by environmental conditions, especially during the early stages. This has been demonstrated experimentally for some species (Gabriel, 1944; Tåning, 1944, 1946, 1952; Martin, 1949; Bailey and Gosline, 1955) and inferred on the basis of observations in nature for many other

3/ According to McHugh, Godsil reported on his findings in a manuscript which was not published owing to Godsil's uncertainty as to the accuracy of the fin-ray counts (which are difficult to make). I have not seen Godsil's manuscript.

species (see Jensen, 1939 for example).

Despite this widely known and accepted fact, the implications with respect to the interpretation of meristic data, for example, seem to be sometimes overlooked or ignored. Only under certain ideal conditions would such environment modified characters be of value. These conditions are: (1) the environmental conditions, and consequently the characters, are not duplicated at different geographical locations. (2) The differences produced are really distinctive. (3) The behavior of the fish is such that the differences are not obscured by mixing (or that the sampling problems are not unduly complicated). Under these ideal conditions such characters would serve as useful "natural tags" with respect to geographic origin of fish, extent of dispersion, migrations and related problems. They would not, however, provide any information on the more fundamental problem of genetic difference.

One has only to consider what is known of sardine spawning to realize how meaningless (from the standpoint of subpopulation characters) meristic characters may be. Aside from the fish which spawn in the bays of Lower California in the fall (and which probably represent a special situation as is discussed elsewhere), sardine spawning is largely confined (for whatever reason) to waters between 13°C and 16.5°C, as measured at a depth of 10 meters. Thus, there is a 3.5° range over which eggs may be deposited and which should lead to meristic differences, at least at the extremes. If the eggs are spawned during a period of no winds, the temperature profile of the water should be fairly constant and the immediate environment of any particular egg should be comparatively unchanging. However, if the temperature profile is stratified (as it would be after an extended period of calm), eggs from a single spawning could be exposed to different temperatures according to their depth distribution.

There is the further situation wherein wind stirring after spawning results in the relatively rapid mixing of stratified water with the subsequent reduction of temperature in the upper part of the stirred layer and an increase in the lower part of the stirred layer. Experiments on other fishes have shown that sudden temperature changes during certain sensitive periods during development can produce large changes in meristic characters.

Thus, it is apparent that, on the one hand, identical environmental conditions may exist at points widely separated in space and time (see above and Ahlstrom, 1954) and that, on the other, eggs from a single spawning may be exposed to a variety of environmental conditions including rapidly changing conditions.

Tagging experiments: Pilchard tagging experiments (using internal tags) were started in British Columbia in 1935, in California in 1936, and in Oregon in 1937. These experiments have been discussed by Brock (1940), Clark and Janssen (1945), Hart (1937, 1938, 1938, 1938, 1938, 1939, 1940, 1941, 1942, 1943, 1943, 1944, 1945, 1945), Hoy (1938), Janssen (1937, 1938, 1938, 1939, 1939), Janssen and Aplin (1945), and Shuman (1939).

The results of most of these experiments have been summarized by Clark and Janssen (1945). They conclude (p. 41):

"Fish tagged by the California Division of Fish and Game in Mexican waters have been retaken in the California fishery off San Diego, San Pedro, Monterey and San Francisco. Sardines tagged in central and southern California have been recovered in the California fisheries and in the Pacific northwest. Fish marked in the Pacific northwest by the Canadian and Oregon governments have been recovered in the California fisheries.

"The rapidity of movement of tagged fish varies with size. The largest sardines will migrate from southern California to British Columbia in five to six months and fish tagged off British Columbia have made the southern migration in the same time interval. Smaller fish do not move as rapidly and several years will elapse before an average-size lot of tagged sardines has spread to distant fishing grounds."

Clark and Marr (1955:32-33) have recently re-examined the tagging data and have given the following summary:

"(a) Fish tagged at any locality from

southern California north to British Columbia were recaptured on all fishing grounds from southern California to British Columbia.... Fish tagged off central and northern Baja California were recovered in large numbers in the southern California catch, moderately in the central California catch and negligibly in the catch of the Pacific Northwest. No tags were recovered from the two lots tagged and released south of Sebastian Viscaino Bay, Baja California (i.e., in Magdalena Bay and south).

"(b) Dispersal of tagged fish throughout the range of the species is difficult to examine in detail owing to lack of recovery facilities in Baja California. However, the available data... show that fish tagged off southern California tended to move more to the north than did fish tagged off central California tend to move south. On the other hand, tags put off British Columbia became increasingly more available to the southern California fishery than to the British Columbia fishery. Fish tagged in southern California were equally distributed on all fishing grounds.

"(c) Fish tagged off Baja California, from Sebastian Viscaino Bay northward, gradually dispersed throughout the California fishing grounds, and there was one recovery off Washington. Over three-fourths of the recoveries were made in the southern California area, however. Owing to the greater distance from the California fishing grounds, the dispersal was slower in the first season after tagging.... than for fish tagged off California. The total number of returns per thousand (56.43 for Baja California, 90.26 for southern California and 79.80 for central California) indicate, however, that in the California fishery the ratio of Baja California fish to California was 56.43/90.26 and 56.43/79.80 and thus the recovery rate of Baja California tagged fish was 60 to 70 percent of the California tagged fish. These percentages reflect the relative availability of the Baja California and California sardines to the U.S.-Canadian fishery.

"(d) The recovery of all tagged fish in southern California in relation to the number of fish taken in the commercial catch was 1.5 times as great as in the central California region.... As noted above, fish tagged off southern California were recovered in all areas in about the same proportion as the total catch and the greater concentration of tagged members in this area came from fish tagged off Baja California. It might be concluded from this that the density of tags was greater in fish tagged off Baja California than in fish tagged off central California, and therefore the population off Baja California was somewhat smaller than that off central California during the period of tagging experiments.

Growth characteristics: Data on the length frequency distributions of sardines have been collected almost from the inception of the fishery. Not until the development of techniques for determining age (Walford and Mosher, 1943a and b), however, was it possible to study growth in any detail. Subsequently, considerable attention has been given to growth and related problems, especially by Phillips (1948), Landa (1953) and Felin (1954).

These studies have shown that there are between-season, between-port and between-year-class differences in "size on age" curves. Similarly, there are within-season, within-port and within-year-class differences. Some of the differences appear to be associated with latitude. Insofar as the characteristics examined are concerned, the sardine population, as sampled by the fishery, is not homogeneous. The nature of the observed differences is not definitely known, but they are probably phenotypic.

Spawning: If all sardine spawning took place at a single time and in a single place, then there would be no subpopulation problem since there would be opportunity for gene flow throughout the population. An important recent addition (Ahlstrom, 1954) to our knowledge of sardines is that there are at least four space-time opportunities for separation between spawning sardines. These include:

1. Southern California offshore area: An area extending roughly from Pt. Conception to Pt. San Quintin and extending some 300 miles offshore. The peak of spawning here occurs in April-May in waters of 13.0°-16.5°C.

2. Lower California offshore area: An

area extending roughly from Pt. Baja to Pt. San Juanico and extending some 200 miles offshore. The peak of spawning in this area is in March-April at temperatures of 13.0°-16.5°C. This area is continuous along a narrow coastal strip with the area off southern California.

3. Lower California inshore area: An area extending roughly from the middle of Sebastian Viscaino Bay to Cape San Lucas and within 100 miles of shore. The peak of spawning in this area is in August-September at temperatures of 18°-23° C.

4. Gulf of California: Very little is known about the distribution of spawning within the Gulf of California, or the temperatures at which it occurs. However, collections made in various years and at different localities show that sardines have spawned throughout the Gulf and that the peak is probably in February-March.

The extent of interchange between these space time groups is not known and is, of course, the critical question with which we are concerned. It is known, however, that in 1952 and 1953 spawning off southern California was negligible; only about 4,000 billion eggs were deposited in this area. But in 1954, some 114,000 billion eggs were spawned off southern California by sardines which must (on the basis of age-composition) have come from the south. One might infer that these fish came from the Lower California offshore area, since the fish in these two localities spawn under approximately the same conditions and since the number spawning in the southern area decreased in that year.

Tissue characteristics: (1) Immunological studies: In recent years there has been increasing use of immunological techniques in the study of blood characteristics with reference to the distinctiveness and relationships of various groups of animals. The most commonly used techniques are the precipitin reaction and the agglutination reaction (see Boyd, 1947, for the description of these and other reactions). The desirable feature of such studies is that it is possible to work with characters known to be genotypic, whereas in morphological studies it is generally difficult or impossible to ascertain how much the genotype is molded or disguised by the phenotype.

Gemeroy (1943), for example, has compared the blood sera of 31 species of fresh and salt water fishes. He found that the relationships demonstrated in this manner in general follow the conclusions based on morphological studies. He concluded, however, that the gulf between species and orders (as presently conceived) of fishes is much greater than it is in birds.

More recently Cushing (1952) has investigated the properties of blood sera of yellowfin tuna (Neothunnus macropterus) and oceanic skipjack (Katsuwonus pelamis) from the central Pacific. He found that at least four distinct blood groups, on the basis of agglutinin content, were present. He also pointed out the possible value of such studies in distinguishing subpopulations within species and the possibility that such differences could serve as natural tags or markers. He has informed me (letter dated February 20, 1953) that, because of the difficulty of securing material among other things, his work will probably not be expanded to include sardines. However, the work has been extended to a number of other species by Cushing and Sprague (1952, 1953).

Rechnitzer (1955) has studied the serological properties (precipitin reaction) of members of the family Enbiotocidae and has found that the relationships thus shown are in agreement with those inferred on the basis of morphology.

(2) Chromotographic studies: The uses to which column chromatography and, more recently, paper partition chromatography have been put are exceedingly diverse (see Stein and Moore, 1951, Zechmeister and Cholnoky, 1941, and Balston and Talbot, 1952 for descriptions and examples of the methods). A recent report by Buzzati-Traverso and Rechnitzer (1953) indicates that chromatograms of fish tissues (muscle) show constancy within species and dissimilarity between species. They also indicate that this technique may be of use in distinguishing subpopulations within a single species.

Such studies of the sardine have been pursued to some extent by Rechnitzer, but his work is still in the exploratory stages.

Farris (ms. "Diet induced variation in the free amino acid complex of Sardinops caerulea") has shown experimentally that sardine muscle amino acids can be modified by diet.

Clearly, much more evaluative, critical work must be done before these techniques are ready to apply to the sardine subpopulation problem in the field

Other attributes: It is possible that studies which are being made for other reasons may fortuitously prove to be of value in recognizing and defining subpopulations of sardines. One such possibility is that the fecundity studies (Clark, 1934), now being extended by MacGregor will detect subpopulations with differing fecundity characteristics, if such exist.

Another possibility is that the fall-spawning sardines, which spawn in waters of above-normal temperatures in Sebastian Viscaino Bay, may prove to belong to a distinct subpopulation.

HYPOTHESIS OF SARDINE SUBPOPULATIONS

From the evidence which is available and which has been reviewed above, three general conclusions may be drawn:

1. Sardines can and do move about through practically the entire range for which evidence is available; i e., from Sebastian Viscaino Bay, Lower California, to the Pacific Northwest. (Little or nothing is known about the movements of sardines to and from southern Lower California and the Gulf of California.) On the other hand, there are pronounced differences in these movements from year to year and also differences in the movements of fish tagged in different localities.

2. Sardines vary between seasons, localities and year-classes with respect to practically all characteristics examined. It is not known whether these characteristics are genotypic or phenotypic, but there is evidence that most, if not all, of them are phenotypic.

3. Information about the distribution of sardine spawning shows that there are opportunities for at least four space-time separations between spawning groups.

What hypothesis about sardine subpopulations can be formulated that is consonant with the available information? At the present state of our knowledge it is possible to advance several alternative hypotheses that are perhaps equally likely to be correct. Instead of advancing a particular hypothesis and testing it, it is possible to ask a more general question, the answer to which will indicate which among the several possible hypotheses is the correct one. This question is. "What interchange is there, if any, between the several space-time spawning groups?" This question may be posed in the affirmative (complete mixing) or the negative (absence of mixing); the method of testing will be the same in either case.

METHODS OF TESTING THE HYPOTHESIS

It is obvious that, regardless of the method used to attack this problem, the crucial test will be the determination of the amount of mixing at spawning time. Complete mixing, lack of mixing or any intermediate condition during the rest of the year are not pertinent to this problem. Therefore, the critical observations must be made on fish collected on the spawning ground. (Of course, if it turns out that there are subpopulations with distinctive characteristics on the several spawning grounds, sampling at other localities will provide information about movements of the subpopulations during the non-spawning season.)

What methods can be used to attack this problem? One method is to collect samples from the several spawning grounds and to examine these to determine if there are any area-specific morphological or meristic differences. But even if there prove to be such differences, the question of whether these are genotypic or phenotypic remains unresolved. This approach, therefore, will not now lead to conclusive results. Inferences may be drawn from certain kinds of information (for example, variation between year-classes produced at a given locality), but conclusive evidence will be provided only by rearing individuals from known parents under different sets of controlled conditions. Unfortunately, methods of rearing pelagic fishes are not yet known.

A second method is to pick characteristics which are known to be genotypic, or which can experimentally be determined to be genotypic, and examine samples from the several spawning grounds with respect to these characteristics. At present, the extent to which any particular characteristic is an expression of the genetic constitution of an individual is unknown. Therefore, if this method is used, it will first be necessary to establish experimentally the genotypic nature of as many characteristics as possible (by the use of chromatographic and/or immunological techniques).

A third method is a tagging experiment, with the tagging and recovering being done on the spawning grounds. Of the several alterna tives, this method would provide the most direct evidence and may have to be employed eventually, regardless of whatever approach is used first. A tagging experiment is, however, a large and costly undertaking, as may be seen from the design described in the appendix. It is therefore more practical to fully assess the possibilities of the second method before reaching a decision about the desirability of a tagging experiment.

SUMMARY

The subpopulation problem in the Pacific sardine is concerned with the number and identity of genetically self-sustaining units within the population.

Evidence of many kinds shows that there are between- and within- season, port and year-class differences. This evidence includes migration, growth, meristic characteristics, etc.

It is not known whether these differences are phenotypic or genotypic; the weight of the evidence is that they are phenotypic.

Of the three possible methods of estimating the exchange between space-time spawning areas, a tagging experiment will yield the most direct evidence.

Tagging experiments are costly, however, and it is more realistic to critically assess the possibilities of chromatography and immunology before reaching a decision about a tagging experiment.

A tagging experiment design is included in an appendix.

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APPENDIX: DESIGN OF A SARDINE TAGGING EXPERIMENT

Purpose and Requirements

A sardine tagging experiment pertinent to the sub-population problem must be designed to answer the question: "What interchange is there, if any, between the space-time spawning groups?" As an aid to the design of such an experiment we are fortunately able to draw upon two sets of information collected annually, primarily for other purposes. First, the location of the spawning areas in space and time are known, and, second, estimates of the numbers of fish spawning in each of these areas are available. (This statement does not apply at present to spawning in the Gulf of California.)

The question posed places two basic requirements upon such an experiment:

1. All tagging must be done on the several spawning grounds at spawning time.

2. All recoveries must be made on the several spawning grounds at spawning time.

It is desirable that several additional conditions be imposed upon the experiment, including:

1. The number of tags put out in each area will be proportional to the size of the population in each area.

2. The recoveries in each area will be proportional to the size of the population in each area.

3. Ten recoveries in a single area from tags put out in any single area is the minimum number that will be accepted.

Two Examples

In an earlier section of this paper, the four possible space-time separations between spawning groups were described. They include (1) the southern California offshore area, (2) the Lower California offshore area, (3) the Lower California inshore area and (4) the Gulf of California. Not enough is known about spawning in the latter area for it to be profitably included in a tagging experiment at this time. (However, two exploratory cruises into the Gulf are planned during 1956.)

Considering only the first three areas, the sardine spawning groups in 1952 and 1953 were distributed approximately in the following proportion:

S. Calif. offshore 5%
L. Calif. offshore 70%
L. Calif. inshore 25%

Now, if 20,000 tags are put out in proportion to the population distribution, then there will be 1,000, 14,000 and 5,000, respectively, tags out in these areas.

With this many tags out, assume, for example, a total spawning population of 2×10^9 fish. If these fish return to the same spawning areas the next season with no mixing or straying

and recoveries are weighted in proportion to the distribution of the population, then it will be necessary to catch about 2,000 tons of fish in order to obtain 200 recoveries (which will satisfy the condition of a minimum of 10 recoveries per area):

Area of tagging and recovery	Catch		Recoveries	
	numbers	tons	number	95% limits
S. Calif. offshore	1×10^6	100	10	5 to 18
L. Calif offshore	14×10^6	1,400	140	117 to 166
L. Calif. inshore	5×10^6	500	50	37 to 66

As indicated, each recovery will be made in the area in which the tag was put out.

But suppose that, instead of no mixing, the fish mix thoroughly between spawning seasons and the fish sort out at random to the three areas in the next season. If the catch is again weighted in proportion to the distribution of the population and a catch sufficient to give 200 recoveries is again made, the recoveries will be distributed as follows:

Area of recovery	Catch		Recoveries		
	numbers	tons	origin	no.	95% limit
s. Calif. offshore	1×10^6	100	s. Calif. offshore	0.5	0 to 4
			L. Calif. offshore	7.0	3 to 14
			L. Calif. inshore	2.5	0+ to 8
				10.0	
L. Calif. offshore	14×10^6	1,400	s. Calif. offshore	7.0	3 to 14
			L. Calif. offshore	98.0	81 to 122
			L. Calif. inshore	35.0	24 to 48
				140.0	
L. Calif. inshore	5×10^6	500	s. Calif. offshore	2.5	0+ to 8
			L. Calif. offshore	35.0	24 to 48
			L. Calif. inshore	12.5	6.5 to 21.5
				50.0	

In this situation, all of the southern California offshore recoveries and the recovery of southern California offshore tags in the Lower California inshore area fall below the minimum requirement of 10. This could be prevented by (1) originally putting out more tags in the southern California offshore area and/or (2) catching more fish (increasing the number of recoveries) in the southern California offshore area.

If the number of tags put out in the southern California offshore area were increased from 1,000 to 4,000, then, with the same catch the recoveries would be:

Area of recovery	Recoveries	
	origin	no.
s. Calif. offshore	s. Calif. offshore	2.0
	L. Calif. offshore	7.0
	L. Calif. inshore	2.5
		11.5
L. Calif. offshore	s. Calif. offshore	28.0
	L. Calif. offshore	98.0
	L. Calif. inshore	35.0
		161.0
L. Calif. inshore	s. Calif. offshore	10.0
	L. Calif. offshore	35.0
	L. Calif. inshore	12.5
		57.5
		230.0

This leaves only the recoveries from all tagging areas in the southern California offshore area below the required minimum. This could be corrected by increasing the catch in the southern California offshore area from 100 to 500 tons.

Area of recovery	Recoveries	
	origin	no.
s. Calif. offshore	s. Calif. offshore	10.0
	L. Calif. offshore	35.0
	L. Calif. inshore	12.5
		57.5

Thus far I have discussed what may be considered the two simple extremes of a somewhat oversimplified model. In one example the tags (20,000) are put out in proportion to the distribution of the population of 2×10^9 fish, which does not change. There is no exchange of fish between areas. The tagged fish are randomly distributed within the population in each area. The recoveries are made in proportion to the population A catch of 2,000 tons is necessary to yield 200 recoveries, which will satisfy the requirement of a minimum of ten recoveries in any single area of fish tagged in any single area.

In the second example, the tags (20,000) are put out in proportion to the distribution of the population of 2×10^9 fish which, again, does not change. There is complete mixing of tagged fish between the three groups and subsequent exchange of tagged fish between areas. The tagged fish are randomly distributed within the population in each area. The recoveries are made in proportion to the distribution of the population. A catch of 2,000 tons will yield 200 recoveries, but in this example the number recovered in some areas does not meet the minimum requirement of 10. In order to achieve this it will be necessary to increase the number of tags put out in the southern California offshore area from 1,000 to 4,000 and also to increase the catch in the same area from 100 to 500 tons (thus increasing the recoveries in that area from 10 to 57.5).

METHOD AND COST

Practically all tagging experiments that have been conducted have depended upon the commercial fishery as a source of fish to tag and as a source of recoveries. In general, such experiments have not been designed to answer the type of question I have posed, but rather to gain information on migrations, mortality rates or population sizes. In fact, considering the fact that most fisheries are not carried out on actively spawning fishes, such experiments could not have been designed to answer the subpopulation question. A notable, and possibly fortuitous, exception is the Pacific herring tagging experiment (Tester, 1949).

The U. S. sardine fishery is similar to most other fisheries in that the location of catch in time and space is different from the location of spawning. Thus, the question of how fish would be caught for tagging and for tag recovery naturally arises. Catching fish for tagging might be accomplished by the use of a lift-net similar to the one used on the M/V Yellowfin (Radovich and Gibbs, 1954). Another and more likely possibility is the use of purse seiners (on a charter basis). The recovery of tagged fish would almost certainly have to be made by seiners because of (1) the quantity involved and (2) the necessity for working offshore in seas too rough to permit operation of the lift-net.

Two problems are involved here. First, is it possible to catch sardines with purse seines on the spawning grounds? There are some indications from the Mexican fishery that this is possible. It would be necessary to firmly establish this (or demonstrate that it cannot be done). Second, granted that the fish could be caught on the spawning grounds in sufficient quantity, how could the tags be recovered at a time when the U. S. processing plants are not ordinarily operating? Special arrangements would have to be made with both U. S. and Mexican plants for their cooperation. Furthermore, it would not be feasible to depend upon magnets in meal plants for the tag recoveries as was done in previous sardine tagging experiments (see Fry, 1937). Instead, it would be desirable to use an electronic metal detector (see Dahlgren, 1936) with which individual tagged fish could be recovered.

The cost estimates for such a tagging experiment are discouragingly high. I estimate that, including the necessary vessel charter, the first year's operation would cost in the order of $60,000. Assuming that feasibility and methods could be reasonably well worked out by the end of the first year, full-scale operations in subsequent years would cost about $120,000 per year.

SOME LIMITATIONS

Despite all the desirable aspects of a tagging experiment, there are limitations. These are, however, limitations of implementation rather than of assumptions or inferences inherent in the method. I indicated above that the examples given are of an oversimplified model, and indeed they are. There are a number of obvious modifications that must be made before the model is reasonably realistic. Foremost among these is an accounting of the loss of tagged fish through mortality and the "dilution" of tags in the total population by the recruitment of a new year-class into the adult population.

If a total mortality rate between years of 50 percent is assumed[2/], then obviously, of the 20,000 tags put out in the initial season, only 10,000 will remain in the first season after tagging. Similarly, the total population will be reduced from 2×10^9 to 1×10^9 fish. If, however, the entering year-class approximates the size of the population to which it is added (again, see Clark and Marr, 1955:22), the total population will again consist of 2×10^9 fish. Thus, while the total population maintains the same size, the number of tagged members has been reduced by one-half. As a consequence, either the number of tags originally put out would have to be doubled or the catch would have to be doubled (or some appropriate combination of increase in the number of tags put out and in catch).

As stated above, a total spawning population size of 2×10^9 fish was assumed in the simple examples given. Any increase in total population size would require a corresponding increase in the number of tags put out and/or the catch. The most recent estimates of total population size (Clark and Marr, 1955:19) indicate a population about four times as large as that used in the examples.

In the discussion thus far the assumption has been made that if there is gene flow throughout the population it is by the mechanism of adults spawning in two or more spawning areas in the same or successive seasons. There is an alternative mechanism; namely, that the fish produced in a given area may themselves spawn in a different area. If the first mechanism is found to obtain, the second may be ignored. If, on the other hand, evidence is negative for the first, then it will be necessary to investigate the second. This will involve determining the smallest size at which sardines may be successfully tagged and being able to associate such fish with an area of origin.

CONCLUSIONS

Of all the methods now known, a tagging experiment is capable of yielding the most definitive answers. However, even in simple situations such an experiment is very costly. And, as the simple examples are made more realistic, all tendencies are toward increasing the magnitude, and thus the cost, of the task.

Until the possibilities of biochemical methods are fully explored, it is reasonable to defer a full-scale tagging experiment. Results obtained by other methods, however, may eventually have to be checked by a full-scale tagging experiment. There is the further possibility that results obtained by other methods may delimit more or less discrete problems which can be resolved by considerably less than a full-scale tagging experiment.

2/ A not unreasonable assumption; see Clark and Marr (1955:26). In this instance it matters not what fraction of the total mortality is from natural causes and what part is from the regular fishery. Tags recovered from the fishery will have no bearing on the solution of the subpopulation problem, although they may be of extreme value in permitting estimates of mortality rates and population size and also in the study of movements.

DISEASE RESISTANT AND SUSCEPTIBLE POPULATIONS OF BROOK TROUT (SALVELINUS FONTINALIS)

By

S. F. Snieszko[1/]

It has been observed that brook trout received from some hatcheries are much more resistant to fish furunculosis caused by Aeromonas salmonicida and to ulcer disease caused by Hemophilus piscium than those obtained from other sources (Wolf, 1954). It will suffice to cite several references to support the assumption that resistance to diseases is a genetic characteristic, the manifestation of which can to som e degree be modified by ecology. Gowen (1952) stated: "Through efforts of workers in the field it is now recognized that agents of diseases and their hosts each are organized and get their characteristics from the development of an inheritance, made up of distinct genes. By specifying the gene for a particular disease reaction it has been possible to show that genes act as protectors from a given disease, as independent of the disease reaction, and as causative agents in the disease syndrome. A clinically manifest disease only results when the proper combination of the genotype of the victim, the genotype of the pathogen, where one is necessary, are properly synchronized with the environment." This thesis has been further developed by Dubos (1954) and Burnet (1953). Therefore, on the basis of the information just given above and the rationale presented in Marr's article in this collection (The Problem of Defining and Recognizing Subpopulations of Fishes) one should feel justified in classifying as populations, or at least subpopulations, strains of brook trout which are kept at separate hatcheries and which differ from each other by significant differences in susceptibility to some infectious diseases.

Furunculosis (McGraw, 1952) and ulcer disease (Snieszko, 1952) are two most destructive bacterial diseases of salmonid fishes. The outbreaks of these diseases among brook trout frequently result in total loss of the infected lots. Because an effective therapeutic control of furunculosis was unknown until 1946 (Gutsell) and of ulcer disease until 1952 (Snieszko, Griffin and Friddle), Embody and Hayford (1925) and Davis (1946) carried out selective breeding of brook trout strains resistant to furunculosis. The results were very encouraging. The strain bred by Embody and Hayford is still maintained at the Hackettstown, N. J. hatchery. Recently Wolf (1954) secured fingerling brook trout from 11 hatcheries and reared them under identical conditions. They were exposed simultaneously to infection with the agents of furunculosis and ulcer disease. At the time the results were prepared for publication, two strains of trout were found to be particularly resistant; one from Hackettstown and the other from the State hatchery at Bellefonte, Pa.

Wolf's work was conducted at the Rome hatchery in northern New York State. In order to reduce the possibility that ecological conditions contributed to the resistance of these two strains of brook trout to ulcer disease and furunculosis, similar experiments were repeated in 1954 and 1955 at the Federal experimental fish hatchery at Leetown, W. Va. Brook trout eggs were obtained from the Federal hatchery at Berlin, N. H., and from the State hatcheries at Bellefonte, Pa., Hackettstown, N. J., and Beaver Creek, Md. Fingerling trout were received from Erwin, Tenn. During incubation and after hatching the fish were maintained under identical conditions of water and nutrition until they were at least half a year old.

The first experiment was started when ulcer disease appeared in the Berlin trout. Some of the diseased Berlin trout were added to trout which originated from the Bellefonte hatchery. Mortalities were recorded and bacteriological examination carried out for 243 days. The

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ultimate losses were 87 percent in the Berlin trout and 51 percent in the Bellefonte fish.

In the second experiment brook trout from Bellefonte, Beaver Creek and Berlin were marked by fin clipping and mixed in equal numbers. Lots were infected by injecting several fishes in each group with a pure culture of H. piscium. Furunculosis broke out spontaneously, and the fish suffered from a mixed infection. Observations were continued for 205 days. Ultimate losses expressed as percentages were as follows: Berlin trout 75, Bellefonte trout 63, Beaver Creek trout 49.

Brook trout from Berlin, N. H., Bellefonte, Pa., and Erwin, Tenn., were compared in the third experiment. The latter are considered to be a local race and are called Appalachian brook trout. (These trout were obtained through the courtesy of Dr. R. E. Lennon of the U.S. Fish and Wildlife Service.)

Somewhat less than half of the fish of all three strains were marked and equal weights (not numbers) per trough were kept together. The remaining fish of each strain were kept separately. All fish were infected by the addition of a mixture of fresh culture of H. piscium and A. salmonicida to the diet fed during the first two days of the test. The experiment was terminated after six weeks.

The test with mixed fish was run in quadruplicate:

	No. per trough				
Source	Lot 1	2	3	4	Total
Erwin	57	64	62	60	243
Berlin	32	32	23	30	117
Bellefonte	34	40	34	40	148
Total	123	136	119	130	508

After six weeks the percentage mortalities were:

	Percent mortality				
Source	Lot 1	2	3	4	Mean mortality
Erwin	90	85	79	97	87.7
Berlin	59	97	83	100	85
Bellefonte	12	12.5	9	17	12.6

The test with the strains kept in separate troughs were run in duplicate. The original numbers per trough and the percentage mortalities after six weeks were:

	No. per trough		
Source	Lot 1	2	total
Erwin	195	210	405
Berlin	69	78	147
Bellefonte	104	116	220

	Percent mortality		Mean mortality
Lot	1	2	
Erwin	97.5	97.5	97.5
Berlin	93	82	87.5
Bellefonte	8.6	16.4	12.5

The results of this experiment will be considered in greater detail elsewhere. Mr. C. E. Dunbar of this laboratory collaborated in the study.

In the light of the results presented above and the general agreement that disease resistance is a function of heredity, there is justification to accept as true populations or subpopulations strains of fishes which display inheritable and significant differences in susceptibility to various diseases. It is a well established fact (Burnet, 1953) that strains of animals resistant to certain diseases originate by natural selection if the disease has an endemic character. It is possible to accelerate the process of the establishment of disease-resistant strains of animals and plants by artificial selection and breeding. It has been found however, that if disease-resistant strains are left under natural conditions, free to mix with susceptible populations, the resistance may gradually disappear in the absence of the pathogen which would destroy the susceptible individuals in such an offspring.

Therefore a practical conclusion can be deduced from the above discussion: Fishes resistant to certain diseases can remain resistant unless exposed to crossing with susceptible individuals.

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NOTE ON THE SUBPOPULATIONS OF LAKE TROUT IN THE GREAT LAKES

By

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The definition and recognition of subpopulations of lake trout (Salvelinus namaycush) in the Great Lakes is a problem of considerable magnitude and interest that has been little investigated. Opportunity for study has become increasingly restricted with the dwindling of populations following the invasion of the sea lamprey (Petromyzon marinus). Lake trout are nearing extinction in Lakes Michigan and Huron; commercial fishery statistics indicate a progressive decline in numbers in Lake Superior. It appears certain that some subpopulations will be or have already been exterminated.

In Lake Superior, where the species probably attains its greatest diversification, different subpopulations are isolated genetically in part by their characteristic spawning seasons and localities. Breeding seasons of different subpopulations (including the siscowet, a form given subspecific ranking, Salvelinus namaycush siscowet) cover a span of at least 6 months (June through November). Some spawn on rocky bottoms in the open lake at depths of less than 20 fathoms; others spawn at 50 to 80 fathoms, apparently sometimes on soft bottoms; still others are reported by Ontario workers to enter streams to spawn, particularly along the east shore of the lake. Tagging studies conducted by both Canadian and United States agencies have revealed a marked tendency of adults to return during successive years to the spawning grounds on which they were tagged.

In addition to differences in spawning seasons and localities, wide variation occurs in size at sexual maturity among different subpopulations. Chemical analyses of flesh samples have shown a large difference in fat content between lake trout and siscowets.

The various subpopulations of lake trout, in Lake Superior at least, are clearly real. Although representatives of some of these may be recognizable by physical characteristics, others unquestionably are not so identifiable. The significance of their existence to the management of the species is great, and there is urgent need for research on various problems which they present.

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